ABSTRACTS

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INVITED SESSION PRESENTATION SUMMARIES

1 OVERVIEW: SCOTT M. HAMMER WORKSHOP FOR NEW INVESTIGATORS AND TRAINEES
Nicolas Chomont1, Serena S. Spudich2
1Université de Montréal, Montréal, Canada, 2Yale University, New Haven, CT, USA
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 cover current knowledge and controversies 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 Dr Stuart Neil on novel aspects of the molecular virology of HIV-1 and SARS-CoV-2. Following this, Dr Guido Silvestri will cover the immune responses against HIV and SARS-CoV-2. Ms Dawn Averitt, an HIV treatment policy advocate and activist will provide a community perspective on the power of community engagement in research. In the following presentation, Dr Monica Gandhi will review novel therapeutic strategies for HIV. Dr Raphael Landovitz will address advances in different strategies for preventing HIV transmission. Dr John Mellors will review advances in preclinical and clinical approaches for functional or sterilizing HIV-1 cure. The workshop will end with an intervention by Dr Rochelle Walensky, who will discuss career opportunities in research and public health. By completing the workshop, attendees will have achieved a head start toward maximizing the knowledge gained and research ideas arising from CROI 2022.

2 SINGLE-CELL ANALYSIS OF THE RNA, PROTEIN, AND GLYCAN FEATURES OF HIV-INFECTED CELLS
Nadia R. Roan
Gladstone Institutes, San Francisco, CA, USA
High-parameter single-cell analysis tools such as CyTOF and scRNAseq have enabled deep examination of HIV-infected cells at an unprecedented level of resolution, and have advanced our understanding of HIV transmission, pathogenesis, and persistence. CyTOF, which enables quantitation of ~40 surface and intracellular proteins on hundreds of thousands of cells at the single-cell level, has revealed insights into the phenotypic features of HIV-infected cells from blood and multiple tissue compartments. CyTOF-Lec, an extension of CyTOF that simultaneously incorporates lanthanide-conjugated antibodies and lectins, has further revealed the glycan features of HIV-infected cells. One key advantage of high-parameter phenotyping of HIV-infected cells is that it enables computational approaches—including testing one named Predicted Precursor as determined by SLIDE (PP-SLIDE)—to distinguish protein and glycan antigens remodeled by infection, from those preferentially expressed on HIV-susceptible cells. PP-SLIDE has further been expanded to predict the original phenotypic features reactivated latent cells prior to ex vivo stimulation, and enabled the identification of surface markers that can enrich for replication-competent reservoir cells from ART-suppressed people with HIV (PWPH). The ability to enrich for unstimulated HIV reservoir cells, along with other technological developments enabling more efficient detection of HIV transcripts, has in turn enabled in-depth multiplexed transcriptomic and proteomic analysis of infected cells from virally-suppressed PWPH by single-cell sequencing-based approaches. Together, these studies have revealed HIV-infected cells to be heterogeneous yet harboring distinct features, including gene expression signatures of cell survival, immune evasion, and cytolysis.

3 SINGLE-CELL MULTIMOMIC ANALYSES OF THE HIV RESERVOIR
Michael R. Betts
University of Pennsylvania, Philadelphia, PA, USA
Recent single cell genomic methodology advances have enabled the measure of HIV DNA within infected cells to move beyond traditional bulk cell HIV DNA viral quantification, sequence intactness, and integration site analysis into the realm of in-depth single cell HIV reservoir studies. This talk will review the constellation of single cell genomic techniques for HIV reservoir characterization based on using the presence of integrated HIV DNA as a molecular tag to identify infected cells. Topics to be covered include a review of what the field has learned so far, remaining outstanding questions, and the practicality of implementing such strategies in the context of HIV cure and eradication studies.
4 SINGLE-CELL MULTIMICS AND EXPANSION DYNAMICS OF HIV RESERVOIR OVER SPACE AND TIME
Ya-Chi Ho
Yale University, New Haven, CT, USA
Understanding how HIV persists over time in different anatomical locations is the key to deciphering mechanisms of HIV persistence, nominating biomarkers, and designing HIV cure strategies. Clinical samples are invaluable resources for understanding HIV persistence in vivo. However, HIV-infected cells are heterogeneous and rare in vivo. Advancement in single-cell technologies resolves the heterogeneity and rarity of HIV-infected cells. Advances in single-cell multi-omics methods profile HIV-infected cells (by HIV DNA and HIV RNA mapping) and respective epigenetic regulator, transcriptome, surface protein markers, and T cell clonality by combining ATAC-seq, RNA-seq, CITE-seq, and T cell receptor (TCR) sequencing within the same single cell. Standard practices such as doublet removal, integration (batch effect correction), cell type annotation, and determining sensitivity and specificity of HIV mapping are required to generate high dimensional single-cell profiles that are reproducible and biologically meaningful. By tracking cells having the same T cell receptor sequences, the immune phenotype of different T cells within the same T cell clone (responding to the same antigen) can be tracked over different time points, at different anatomical locations, and before and after antigen stimulation. By examining the transcriptome of frozen tissues in situ without making cellular suspensions, called spatial transcriptomics, cell-cell interactions in tissue microenvironments can be examined at 55 µm resolution down to 10 µm and even single-cell resolution. Expanding from spatial transcriptomics, spatial ATAC-seq and spatial protein phenotyping provide additional understanding of cellular states in situ. The main challenge of using these advanced methods in HIV reservoir profiling remains to be the rarity of HIV-infected cells. Overall, advanced single cell technologies, with careful bioinformatic analysis and biological validations, revolutionized our understanding of HIV-infected cells down to the single cell level over space and time.

5 NOVEL APPROACHES TO CHARACTERIZING IMMUNE RECOGNITION
Michael Birnbaum
Massachusetts Institute of Technology, Cambridge, MA, USA
The adaptive immune system is breathtakingly complex. B and T cells both rely upon somatically recombined antigen receptors with theoretical diversity of 10^18 possible combinations to recognize the host of possible pathogens or transformed cells that may be encountered. While this complexity is key to proper immune function, it also complicates its study. A comprehensive understanding of T and B cell recognition can aid in understanding how immune responses succeed or fail, how to generate better predictive computational models, and how to design the next generation of therapies. In this presentation, I will introduce adaptive immune recognition, and provide an overview of both established and emerging technologies to interrogate what the immune system sees.

6 FULLMINANT HEPATITIS IN CHILDREN
Luz Helena Gutierrez Sanchez
University of Alabama at Birmingham, Birmingham, AL, USA
Fulminant hepatitis in children is a dynamic and life-threatening condition with a broad differential diagnosis. Diagnosis is age dependent and therefore evaluation of patients presenting with hepatitis should be tailored to age. Fulminant hepatitis in children (majority of them below 5 years in age) with high frequency of human adenovirus viremia, more specifically human adenovirus type 41. Further investigations have shown presence of adeno-associated virus 2. Pathophysiology of etiology is unclear, as tissue samples have not shown direct viral cytopathology. Possible etiology for the increased number in cases of fulminant hepatitis remains unclear at this time.

7 HBV PREVENTION: NEWER VACCINES AND THE BOUNDARIES OF HBV PROTECTION
H. Nina Kim
University of Washington, Seattle, WA, USA
After participating in this session, attendees will be able to
- Describe the newer options for hepatitis B immunization and their seroprotective efficacy in key subpopulations.
- Summarize our current understanding of the role of antiviral therapy in HBV prevention.
- Identify the current gaps in knowledge related to HBV immunity in the patient with HIV and an isolated hepatitis B core antibody profile.

8 HEPATITIS DELTA INFECTION AMONG PERSONS LIVING WITH HIV
Charles Béguelin
University of Bern, Bern, Switzerland
Approximately 5-10% of individuals with hepatitis B surface antigen (HBsAg) are coinfected with the hepatitis delta virus (HDV), totaling 15-60 million people worldwide. The prevalence of HDV-coinfection varies considerably across clinical settings, geographic areas, and the constellation of risk factors within study populations. Injection drug use is a well-known route of transmission for HDV and probably the main one in high-income countries, whereas other routes of transmission remain less clear, with high-risk sexual behaviors being suggested as one. Epidemiological data on HDV infection among persons living with HIV (PLWH) remain limited as the uptake of routine testing is generally suboptimal due to the lack of awareness, issues with standardizing diagnostic techniques and limited treatment options. Hepatitis delta is the most severe form of viral hepatitis: a faster progression to liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) has been described in populations with and without HIV. The magnitude of HDV replication seems to be an important driver of morbidity.

In this workshop we will discuss testing recommendations for Hepatitis B and delta, the natural history of HBV/HDV coinfection, new treatment of hepatitis delta and how to monitor treatment success, as well as screening indications for HCC.

9 ADAPTIVE PLATFORM TRIALS: EXPERIENCE FROM THE COVID-19 PANDEMIC
Michael D. Hughes
Harvard T.H. Chan School of Public Health, Boston, MA, USA
Adaptive platform trials are randomized clinical trial designs that enable the evaluation of multiple interventions in a single, highly standardized trial framework undertaken using a master protocol. Fundamental to these designs is allowance for interventions to be added during the course of the study, sharing of a control group in evaluating multiple interventions in parallel, and decision criteria for dropping interventions for futility or efficacy based on interim analyses. Other more complex adaptations may also be incorporated, such as changing allocation ratios across randomized arms in response to interim results, seamless phase II to III transitions, and sample size re-estimation. A large number of platform trials were initiated in response to the COVID-19 pandemic. I will review some key features of platform trial designs with illustrations from the COVID-19 arena, and discuss advantages and challenges of using them in comparison to traditional randomized trials.

10 UNPACKING MODELING STUDIES
Viviane D. Lima
British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada
There is increasing recognition of the usefulness of mathematical models in informing public health intervention strategies and policymakers on how to address different epidemics. Over the years, we have seen many models testing different interventions to prevent the spread of HIV. Lately, mathematical models have been used to assess interventions focused on controlling the spread of COVID-19 and, more recently, of Mpxo. However, as we will show in this presentation, we saw considerable pressure to fast produce mathematical models for COVID-19 to inform on different policies to control the spread of this disease. Consequently, we saw that several of these models missed the mark, and others, although not perfect, were useful in making predictions based on different interventions. In this presentation, I will describe how mathematical models have informed public health interventions. We will provide an overview of the use of mathematical models to prevent HIV, COVID-19 and Mpxo. I will propose a list of criteria to evaluate and write publications involving mathematical models. Finally, I will discuss some of the lessons learned for properly using mathematical models and interpreting results and propose a pathway moving forward.
11 SOCIAL AND BEHAVIORAL SCIENCE: THE MISSING INGREDIENT TO SUCCESSFUL CLINICAL TRIALS
Heidi van Rooyen
Human Sciences Research Council, Cape Town, South Africa
This talk considers the role of social and behavioral science at every stage of the clinical trial process from design to enrolment, participation, retention and outcomes. Based on a review of the literature and three decades’ experience as a social scientist conducting leading HIV and COVID studies, it argues that understanding human behavior and decision-making alongside the context in which these decisions are made are key to effective, efficient and quality clinical trials.

12 MODELING THE DYNAMICS OF HIV INFECTION: ESTABLISHING PARADIGMS FOR TREATMENT AND CURE
Alan S. Perelson
Los Alamos National Laboratory, Los Alamos, NM, USA
Although mathematics and physics are commonly thought of as being abstract and not of much practical use particularly in medicine, theoretical ideas expressed mathematically changed the way we think about HIV infection and its treatment. I will show how this approach when used to analyze clinical data led to fundamental insights about HIV and its successful control with drug therapy. The challenge now is to cure HIV. I will discuss current mathematical and experimental approaches that suggest the immune system can be manipulated for this task.

13 HIV AND GLOBAL HEALTH IN A PANDEMIC ERA
Kevin M. De Cock
Centers for Disease Control and Prevention (former), Nairobi, Nairobi Area, Kenya
Four broad themes run through this year’s N’galy–Mann lecture: clinical medicine, HIV, health security, and global health. Three patterns of disease characterized medicine in East Africa at the time that AIDS was first described in the United States: diseases of poverty, mainly infectious; non-communicable diseases with differing international epidemiology; and classic tropical diseases restricted in distribution by ecologic needs of parasites and vectors. Limited resources did not prevent the practice of good medicine under adverse circumstances, nor application of basic principles of research. The recognition of a second AIDS virus (HIV-2) in West Africa in the mid-late 1980s required applied research to assess implications and potential global impact of this novel infection. CDC established a second collaborative research site in sub-Saharan Africa, Projet RETRO-CI, in Abidjan, Cote d’Ivoire (the first was Projet SIDA in the Democratic Republic of Congo, where N’Galy and Mann made seminal contributions). Controversy around HIV-2 diagnosis, transmission, and pathogenesis was slowly resolved through West African research showing HIV-2 was an AIDS-causing pathogen, slower than HIV-1 in its progression, and less transmissible until late in the course of infection. Mother-to-child transmission was exceptionally rare. Claims that HIV-2 protected against HIV-1 were not substantiated. Projet RETRO-CI clarified the spectrum of HIV-associated disease and the dominant role of tuberculosis. Placebo-controlled trials demonstrated efficacy of short-course zidovudine for prevention of perinatal transmission of HIV-1, and of cotrimoxazole prophylaxis in reducing hospitalization and mortality in persons with HIV. Global health today is dominated by discourse around health security. The West African and Congolese Ebola epidemics since 2014 aroused strong declarations, yet the world was poorly prepared to address the pandemic of COVID-19. Health in the world has changed substantially since AIDS emerged. As 2030, the year for delivery on the Sustainable Development Goals, approaches, development assistance for health remains essential to address traditional, unfinished commitments yet does not match today’s global burden of disease. COVID attendees are encouraged to remember colleagues lost to COVID-19 and other challenges; to assess priorities in today’s global health, including relating to HIV; and to reflect on what issues N’Galy and Mann would focus on today.

14 COMMUNITY AND ADVOCATES ARE EQUAL PARTNERS IN RESEARCH AND DEVELOPMENT
Yvette A. Raphael
Advocacy for Prevention of HIV and AIDS, Midrand, Gauteng, South Africa
Yvette Raphael will take the audience on a journey, sharing how cisgender women, and their allies, organized in Africa and across the world, to go beyond simply being study participants to being research leaders in the fight for a future free of new infections. Community leadership is not nice to have, and it is not performative. It is an imperative, and advocates must be equal partners in the effort. Vulnerable communities and populations must always be centered as we work collaboratively, transparently, and equally side-by-side with governmental agencies, researchers, product developers, policymakers, and funders, among others. The only way we end the epidemic is with leadership coming from communities, including Black Women in Africa and around the world.

15 20 YEARS OF PEPFAR: LOOKING BACK, STRIDING FORWARD
John Nkengasong
US Department of State, Washington, DC, USA
We are closer to ending HIV/AIDS as a public health threat. During its first 20 years, PEPFAR has worked with partner-country governments and other stakeholders, including the Global Fund to Fight AIDS, Tuberculosis and Malaria, UNAIDS, communities, and other partners to end HIV/AIDS as a public health threat by 2030. Thanks to global and local HIV stakeholders, according to UNAIDS, AIDS-related deaths have been cut by 64% since their peak in 2004 and new infections have been reduced by 42% through the delivery of essential prevention and treatment services. Globally, 73% of people living with HIV are accessing antiretroviral therapy (ART) and 20 million of them are supported by PEPFAR worldwide. PEPFAR has helped prevent HIV infection in men and boys, including by supporting 30 million voluntary medical male circumcisions in east and Southern Africa. Since 2015, new HIV diagnoses among adolescent girls and young women have declined in all geographic areas implementing the PEPFAR-led DREAMS (Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe) public-private partnership. This year, the program provided critical care and support for 7 million orphans, vulnerable children, and their caregivers so they can survive and thrive, testing for over 64 million people and over a million received PrEP. Through PMTCT and comprehensive programming, PEPFAR’s PMTCT programs enabled 5.5 million babies to be born HIV-free to mothers living with HIV. Finally, PEPFAR’s investments also strengthened the systems that drive effective, efficient, and sustainable health care. PEPFAR has helped train 340,000 health care workers to deliver and improve HIV care and other health services, creating a lasting health system for partner countries to confront other current and future health challenges.

16 THE PATH TO HEPATITIS C CURE
Anna S. Lok
University of Michigan, Ann Arbor, MI, USA
HBV infection remains a major global health burden, with 1.5 million new infections, 296 million chronically infected persons, and 820,000 HBV-related deaths in 2019. Current treatment with pegylated interferon alfa (pegIFNa) or nucleos(t)ide analogues (NAs) can suppress HBV DNA replication, decrease liver inflammation, reverse liver fibrosis and decrease risk of cirrhosis, hepatocellular carcinoma and liver-related deaths. However, HBsAg loss rarely occurs. It is generally accepted that sterilizing cure is not feasible, thus, the goal is to achieve functional cure defined as sustained clearance of HBsAg and HBV DNA after completing a finite course of treatment. Functional cure will require silencing of cccDNA and integrated HBV DNA and restoration of immune response to HBV. Combination therapy of direct-acting antiviral drugs and immune modulatory therapies will be necessary to completely suppress HBV replication, decrease HBsAg production to very low levels, and activate or remove blockade of HBV-specific immune response. Suppression of HBV replication may involve combinations of NA, capsid assembly modulators (CAMs), and entry inhibitors, though translation inhibitors such as small interfering RNAs (siRNA) and antisense oligonucleotides (ASO) may contribute. Decrease in HBsAg production may involve combinations of entry inhibitors, translation inhibitors - siRNA/ASO, and drugs that block release of HBV or viral proteins such as nucleic acid polymers (NAP). Combination of CAMs and NAs result in deeper suppression of HBV DNA replication but has minimal effect on HBsAg or HBsAg production and withdrawal of therapy leads to near universal viral relapse. siRNA/ASO with or without NAs lead to marked decrease in HBsAg production but rates of HBsAg loss remain low with strategies tested so far. To date, clinical trials of immune modulatory therapies have met with limited success. New approaches including combination of therapeutic vaccines and check point inhibitors, and HBV antibodies aimed to neutralize HBV and to enhance antigen presentation. So far, few of the new therapies have resulted in HBsAg loss at the end of treatment.
and even fewer have resulted in off-treatment HBsAg loss. In addition to new drugs, development of standardized assays of new HBV markers to confirm target engagement, assess cccDNA transcription, and to confirm "cure" need to occur in parallel. Availability of assays to measure recovery of HBV-specific immune response would help identify which patients need additional immune modulatory therapies. Platform trials should be deployed to improve efficiency. Safety remains a high priority given the excellent safety profile of NAs. While the goal of achieving functional HBV cure in a high proportion of patients remains remote, the efforts invested in the last 5 years have provided important tools and insights towards achieving that goal.

17 ORIGINS OF HIV-1 AND SARS-CoV-2
Michael Worobey
University of Arizona, Tucson, AZ, USA
Dr. Worobey will discuss the scientific evidence for when, where and how both the HIV/AIDS pandemic and the COVID-19 pandemic originated, and what we can learn from this knowledge to prevent or mitigate future pandemics. In both cases, cross-species transmission into humans via wildlife consumption, versus via laboratory accident, were plausible hypotheses of origin. And in both cases, there is now overwhelming evidence in favor of the “natural” zoonosis route. Indeed, in the case of COVID-19, we have insights into the genesis of the pandemic that are in many ways unparalleled in the history of investigating pandemic origins.

18 FROM PROMISE TO REALIZATION: SEQUENCING AND SURVEILLANCE FROM HIV-1 TO SARS-CoV-2
Emma B. Hodcroft
University of Bern, Basel, Basel-Stadt, Switzerland
The advent of cheaper viral sequencing and opportunity to offer customized treatment through identification of resistance mutations in patients with HIV-1, also offered the first large-scale opportunity to use sequencing to generate insights into a global infectious disease pandemic. Using HIV-1 sequences, scientists were able to track mutations globally and within countries and use them to gain groundbreaking understanding of virus transmission and the evolution of resistance. Though invaluable in contributing to our knowledge of virus dynamics, much of what was feasible with HIV-1 was difficult to extend to other viruses due to the challenges and expense of full-genome sequences and the difficulty of obtaining samples from acute infections. More recent advances have made next-generation sequencing (NGS) possible and affordable, and growing realization of the insights sequencing can contribute has increased interest in generating sequences for an increasing variety of viruses. Against this backdrop of an advent of a new age of genomics in viral research, the SARS-CoV-2 pandemic has thrown sequencing and phylogenetics into the limelight, allowing the collection and sequencing of more samples than could even be conceived prior to 2020. It’s an opportune time to consider not only where we’ve come from, but how the promise of 14 million sequences has been realized, and what the future holds for sequence-enabled pathogen research.

19 ANTIBODY-DRIVEN EVOLUTION OF HIV-1 AND SARS-CoV-2
Paul D. Bieniasz
Rockefeller University, New York, NY, USA
In this presentation, Dr. Paul Bieniasz will discuss antibody-driven evolution of HIV-1 and SARS-CoV-2.

20 THE LANDSCAPE OF ADHERENCE TESTING
Peter L. Anderson
University of Colorado Anschutz Medical Campus, Aurora, CO, USA
Inadequate adherence to modern ART is commonly defined as <80% of doses taken, and that for daily PrEP depends on route of exposure: <4 doses per week on average for men and <6-7 doses per week on average for women. Inadequate ART adherence is common, estimated at ~40% in the USA and that for daily PrEP is as high as 30%-90% depending on the population and study. This underscores the need for accurate and precise adherence measurement tools to help interpret study results and manage patient care. This presentation will review the current landscape of objective adherence measurement tools with a focus on drug concentrations in blood, urine, and hair. Additionally, important considerations for using adherence measurements will be reviewed including the importance of aligning adherence measurements with intentional versus unintentional nonadherence. Finally, the application of adherence measurements to understand pharmacokinetic forgiveness, clinical trial efficacy, and adherence-response relationships will be discussed.

21 DIGITAL TECHNOLOGIES FOR SUPPORTING ADHERENCE
Lisa Hightow-Weidman
Florida State University, Tallahassee, FL, USA
It is estimated that adherence to chronic medications is approximately 50%, resulting in an annual cost of at least 500 billion dollars in the United States (US) alone (e.g., ~16% of the total US health care expenditures). Further, medication nonadherence can affect overall healthcare utilization, quality and length of life, and health outcomes. To date, there is no “gold standard” measure of adherence. Thus, adherence measurements that are easy to operationalize, provide valid and complete data and are not cost prohibitive are greatly needed. Digital technologies can provide both direct and indirect measures of adherence and have the potential to allow for both adherence monitoring and intervention delivery through provision of “real-time” information and through direct connections to providers. Video observed therapy (VOT) has been conditionally recommended by the WHO as an alternative to directly observed therapy (DOT) and can be delivered both synchronously and asynchronously. Ingestible biosensors (e.g., digital pills) have been shown to be both feasible and acceptable across multiple disease states, though additional research is needed prior to broad-scale deployment. Tools that leverage automation, artificial intelligence (AI) and machine learning can aid in the prediction of nonadherence and provide timely prompts and tailored reminders, thus offering the potential for delivery of personalized medicine. A brief review on how digital technologies can be utilized to support adherence, through both measurement and intervention will be provided. The advantages and limitations of currently available digital technologies for measuring adherence will be discussed, highlighting the unique characteristics and performance of these tools in the context of intervention trials to improve adherence outcomes. Practical strategies, best practices, challenges, and future opportunities will be presented.

22 CONSIDERATIONS FOR MEASURING ADHERENCE WITH LA/ED THERAPIES IN LOW- AND MIDDLE-INCOME
Catherine Oreilly
Desmond Tutu Health Foundation, Cape Town, South Africa
Newer long acting (LA) and extended duration formulations are becoming available for the treatment of HIV, removing the need for daily tablet taking. Most data currently available are for those already suppressed on oral daily ART (switch studies). As resource limited settings move towards registration of the first of these LA preparations, we explore who live with HIV might be the best fit for the use of LA/ED preparations in a RLS; and discuss health system considerations in monitoring adherence during the roll out these undoubtedly useful products, which might require more clinic visits and staff skill.

23 FROM EFFICACY TO EFFECTIVENESS: CATALYZING ROLLOUT OF LONG ACTING PREP
Nyaradzo M. Mgodi
University of Zimbabwe, Harare, Zimbabwe
Translating data from clinical trials into policy and clinical practice. Barriers and facilitators for implementation. Lessons learnt from oral PrEP rollout. Challenges unique to LMIC.

24 INEVITABLE INEQUALITIES: WHY ME KEEP MAKING THE SAME MISTAKES AND HOW WE CAN STOP IT
Laron E. Nelson
Yale University, New Haven, CT, USA
The discovery of the effectiveness of long-acting injectable agents for HIV pre-exposure prophylaxis holds great promise for accelerating an end to the epidemic in the United States and getting to zero new HIV infections globally. Nonetheless, inequalities that currently persist despite the introduction of daily oral PrEP agents offer important lessons about the potential of new innovations to widen racial, ethnic and gender disparities in HIV incidence. This presentation will discuss the pre-existing interlocking social, structural and policy impediments that threaten to undermine equity in the prevention impact of long-acting injectable agents for PrEP as well as discuss approaches to optimize progress towards achieving global HIV prevention targets by avoiding the repeat of past mistakes.
LA PrEP: WHAT WE KNOW AND WHAT WE STILL NEED TO KNOW
Sunil S. Solomon
The Johns Hopkins University School of Medicine, Baltimore, MD, USA
The findings of the two Phase II long-acting cabotegravir pre-exposure prophylaxis trials (HPTN 083 and 084) have heralded a new era of HIV prevention. However, demonstration of efficacy in select populations is only the first step. To truly capitalize upon the potential of LA PrEP, it is imperative that these Phase 3 trials be translated to real-world settings to ensure access to all populations vulnerable to HIV infection. Furthermore, we do not necessarily know the best ways to deliver LA PrEP in populations that might benefit the most. This presentation will provide an overview of gaps in data ranging from efficacy to acceptability at the individual-, provider- and structural-levels that may be required to usher in a new era of HIV prevention - one that includes LA cabotegravir for PrEP.

HIV RESERVOIRS: OBSTACLES TO A CURE
Janet M. Siliciano
The Johns Hopkins University School of Medicine, Baltimore, MD, USA
The major barrier to curing HIV infection is a latent reservoir in resting CD4+ T cells that was initially demonstrated with a quantitative viral outgrowth assay (QVOA) in which T cell activation is used to reverse latency and allow exponential viral outgrowth. In persons living with HIV (PLWH) who are on suppressive antiretroviral therapy (ART), decay of this reservoir is so slow that lifetime persistence is guaranteed. Recent studies of HIV integration sites suggest that selection against inducible proviruses gradually transforms the reservoir to a non-inducible state of deep latency. However, our recent QVOA studies of PLWH on ART for 20 years show no decrease in inducible, replication-competent proviruses. This finding was confirmed with the intact proviral DNA assay (IPDA), a digital droplet PCR assay that excludes most of the defective proviruses that complicate reservoir measurements. Defective proviruses can be excluded by the QVOA, the IPDA, and by sequencing based assays. However, the latter are non-quantitative due to inefficiencies of the long-distance PCRs involved. The reason that the reservoir of inducible, replication-competent proviruses does not continue to decay is that latently infected cells can proliferate following encounter with antigen. Activation of CD4+ T cells through the antigen receptor can reverse latency, exposing cells to viral cytopathic effects and immune clearance. However, cells can also proliferate without latency reversal, allowing generation of large clones of infected cells that still retain the potential to produce virus following a subsequent stimulation. Rebound upon treatment interruption can come from large clones or minor variants representing less than 1% of the reservoir. This, preventing rebound requires reduction of the reservoir by many logs, a daunting proposition given that no clear reductions have been proven in clinical trials. A critical factor in rebound is whether the viruses that are induced are resistant to antiretroviral drugs. The QVOA, IPDA, and sequencing based assays, however, do not detect non-replicating latently infected cells that can proliferate following reexposure to antigen.

RESTRICTIONS ON REPRODUCTIVE RIGHTS AND THEIR IMPACT ON PEOPLE LIVING WITH HIV
Denise J. Jamieson
Emory University, Atlanta, GA, USA
This presentation will review how recent legislative actions in the United States have restricted the reproductive rights of people living with HIV. Specifically, we will use the Dobbs v. Jackson Supreme Court decision and subsequent state restrictions as a U.S. case study utilizing specific clinical scenarios and conclude with larger global considerations based on the US example of how abortion restrictions may impact health.

APPROACHES TO PEDIATRIC CURE
Philip J.R. Goulder
University of Oxford, Oxford, England, United Kingdom
Major differences exist between HIV infection in children and adults which impact both on cure potential and on the approach to achieving cure. The tolerogenic, highly regulated, early-life immune environment is associated with weak antiviral immunity and rapid HIV disease progression in cART-naive children, but the benefits of low-level immune activation may outweigh the disadvantages in increasing cure potential among early-cART treated children. The combination of early-life immunity and very early initiation of cART in children results in small, low-diversity viral reservoirs that diminish rapidly to undetectable levels. The second major difference between pediatric and adult infection is viral transmission from the mother. In mother-to-child transmission, the transmitted virus has relatively low replicative capacity, which is associated with low reservoir size in the recipient. There are also striking immune sex differences in early life that increase female fetal susceptibility to MTCT and that appear to decrease cure potential among females, at least in infancy. This presentation will discuss the factors that are currently believed increase cure potential among children and, based on this, the strategies being adopted now and that may be adopted in the future to facilitate pediatric cure.

NONHUMAN PRIMATE STUDIES OF PEDIATRIC CURE INTERVENTIONS
Ann Chahroudi
Emory University School of Medicine, Atlanta, GA, USA
Interventions to reduce HIV reservoirs and prevent viral rebound in the absence of ART would be highly beneficial for the 1.7 million children living with HIV. Pediatric nonhuman primate models have informed our understanding of viral persistence following perinatal infection as well as the safety and efficacy of cure strategies. Infant rhesus macaques can be infected with SHIV or SHIV strains by experimental infection of dams during pregnancy, inoculation of amniotic fluid, or, more commonly, through oral exposure in the first days of life to simulate intrapartum swallowed blood and/or vaginal secretions or later in infancy to model breast milk transmission. Antiretroviral therapy (ART), most commonly in the form of a single subcutaneous injection of two reverse transcriptase inhibitors and one integrase inhibitor given daily, can then be used to suppress viral replication. Approaches to prevent reservoir establishment, reverse viral latency, clear infected cells, and enhance immune control of virus replication have been tested and results of these studies will be reviewed. Specific cure approaches evaluated in infant nonhuman primate models include polyfunctional antibodies, including those with broadly neutralizing capacity, therapeutic vaccines, Toll-Like Receptor (TLR) agonists, and a non-canonical NF-κB pathway activator. This body of work has highlighted important distinctions in responses to interventions in pediatric vs. adult systems, highlighting the importance of targeted studies at different ages. Finally, ideas for future research in infant nonhuman primates designed to inform cure-directed clinical trials for children living with HIV will be discussed.

PROGRESS IN CLINICAL TRIALS OF PEDIATRIC HIV CURE
Roger L. Shapiro
Harvard University, Boston, MA, USA
Children living with HIV are ideal candidates for strategies that may lead to post-treatment control following antiretroviral treatment (ART), and those treated from very early life may have unique features that are especially favorable for cure research. With early effective ART, the latent intact HIV reservoir is drastically reduced as the immune system matures through early life. Such children may have the potential for post-treatment control through the outright elimination of intact virus, its elimination from encoding regions of the genome, or immune control mechanisms. This talk will focus on approaches to pediatric cure that include early ART, the use of broadly neutralizing monoclonal antibodies (bNAbs), and the potential for vaccine-based therapies that may promote post-treatment viral control. The talk will review key studies and recent clinical trials, and opportunities for novel approaches. New data for reservoir dynamics and reservoir markers specific to the pediatric population will be discussed, and the talk will address some of the challenges of performing pediatric cure research (including the need for analytic treatment interruption studies and access to new agents) as well as the unique opportunities to understand avenues to cure offered by pediatric trials.

FUTURE DIRECTIONS IN OUTPATIENT THERAPY FOR MILD TO MODERATE COVID-19
Kara W. Chew
University of California, Los Angeles, Los Angeles, CA, USA
Robust efforts to rapidly develop outpatient therapies for acute COVID-19 leveraged existing platforms and small molecule antivirals originally developed for other viral infections to rapidly identify multiple effective therapies that reduce risk for hospitalization and death in persons at increased risk for severe COVID-19 and have been authorized for this use. These have included single and combination anti-SARS-CoV-2 monoclonal antibodies (mAbs),
remdesivir, nirmatrelvir/ritonavir, molnupiravir, and convalescent plasma in select populations. However, the limitations of mAbs became evident early, and none are currently authorized for use in the U.S. The remaining available therapies each have limitations, such as drug-drug interactions, challenges with administration, or uncertain and potentially lower efficacy. In this presentation, we will discuss the evidence for antiviral therapy for mild-to-moderate COVID-19 — who should be treated? — in today’s context of vaccinations, prior infections, and lower hospitalization and death rates. We will also discuss selection of therapy for immunocompromised persons and touch on the COVID-19 therapeutics pipeline and current challenges in outpatient COVID-19 clinical trial design.

VIRAL REBOUND DURING AND AFTER SARS-CoV-2 TREATMENT
Jonathan Z. Li
Harvard Medical School, Cambridge, MA, USA
SARS-CoV-2 viral rebound after treatment with nirmatrelvir-ritonavir is an area of ongoing concern for both patients and providers. However, viral rebound has been described both during and after treatment with other antiviral therapies as well. It also appears that viral and symptom rebound can occur in the absence of antiviral therapy, especially in the immunosuppressed patient population. In this presentation, we will review the pathogenesis and risk factors for viral rebound during and after SARS-CoV-2 treatment. We will also explore the clinical and transmission risk of viral rebound and potential management strategies.

WHAT WE KNOW NOW ABOUT LONG COVID SYNDROMES
Michael J. Peluso
University of California San Francisco, San Francisco, CA, USA
It is now widely accepted that SARS-CoV-2 infection can affect long-term health and quality of life. Long COVID, a type of post-acute sequelae of SARS-CoV-2 infection (PASC) characterized by persistent unexplained symptoms, has a major impact on the health of many COVID-19 survivors. Although many individuals (up to 30%) experience some limited symptoms in the weeks and months following COVID-19, the prevalence of severe disabling Long COVID is less common (perhaps <5%). Long COVID syndromes are variable and include general (e.g., fatigue) and organ-specific symptoms (e.g., shortness of breath, palpitations, neurocognitive symptoms), as well as symptoms resembling other medically unexplained syndromes (e.g., myalgic encephalomyelitis/chronic fatigue syndrome, dysautonomia, post-exertional malaise). For reasons not yet understood, female sex is a strong predictor of Long COVID, as is the presence of certain comorbidities, particularly obesity. Mechanisms that might plausibly contribute to Long COVID include irreversible tissue damage associated with acute infection, persistence of SARS-CoV-2 antigen or possibly a viral reservoir, residual or ongoing immune activation and inflammation, reactivation of other latent human viruses, microvascular dysregulation and thrombotic events, microbial translocation, dysbiosis, and autoimmune phenomena. These mechanisms may act in isolation or in combination to drive Long COVID syndromes. Notably, many if not all of these pathways have been implicated as possible mechanisms for the excess rate of cardiovascular disease and other comorbidities in people living with HIV. Industry engagement in Long COVID research is growing, and NIH funding for clinical trials is emerging through programs such as the RECOVER Initiative. As a result, we are entering an era of experimental medicine, in which potential interventions will be used as tools to probe the biology of the disease. This presentation will provide an overview of the proposed biological mechanisms contributing to Long COVID, with a focus on the current state of evidence, human and animal models, and the emerging therapeutic agenda.

IMPLEMENTING THE PrEP AND PEP TOOLKIT
Colleen Kelley
Emory University, Atlanta, GA, USA
Since FDA approval of daily, oral FTC/TDF for HIV pre-exposure prophylaxis (PrEP) in 2012, the HIV prevention field has been diligently working on the development of more options for biomedical interventions to empower users with choice of prevention products. While the metaphorical HIV prevention ‘toolkit’ remains sparsely filled, some people can now benefit from the use of on-demand oral FTC/TDF or injectable, long-acting cabotegravir for HIV prevention in addition to daily, oral FTC/TDF or FTC/TAF. However, stark racial, gender, and global disparities in PrEP use and significant implementation challenges are hindering the true potential of PrEP to reduce HIV incidence for all populations and achieve the US Ending the HIV Epidemic initiative goals. For clinical providers of PrEP, access to post-exposure prophylaxis (PEP) services, supporting persistence on PrEP, implementing new PrEP follow-up care guidelines, and providing holistic sexual health services are evolving components of cutting-edge PrEP care delivery. In this talk, we will review the current landscape of PrEP and PEP options, identify challenges to wide-spread uptake, summarize current best practices for implementing the PrEP and PEP toolkit, and identify outstanding questions for future research.

PREVENTION OF STIs IN ADULTS AND ADOLESCENTS A SEXUAL REPRODUCTIVE HEALTH PERSPECTIVE
James Kiarie
World Health Organization, Geneva, Switzerland
Sustained scale up of STI prevention requires an approach that comprehensively addresses the reproductive health, sexual health and wellbeing of affected and at-risk adults and adolescents. Beyond upcoming interventions such as doxyPEP and vaccines that this presentation will focus on, they will be discussed in the context of the entire prevention armamentarium including socio behavioral change, condoms, screening and treatment.

The impact of STI prevention interventions will be determined not only by their efficacy but also by a cascade of need, demand, uptake and adherence as seen in other sexual reproductive health areas such as a contraception, antenatal care and HIV PrEP. Programs to prevent STIs will need to draw on lessons from these areas for rights-based approaches to address each level of this cascade in the context of client choice. It will be important to consider socio, economic and health system contexts for sustainability and scale up of the prevention of STIs. In this session we will also discuss what is still unknown and where STI prevention needs to go. This will include concerns regarding antimicrobial resistance, the state of evidence and research, appropriateness of the interventions for different populations and STIs, systematic screening, and controversies in the field.

MPOX PREVENTION
Jade Ghosn
University of Paris Cité, Paris, France
Since May 2022, dozens of countries worldwide have reported cases of mpox in individuals with no link with endemic countries in Africa. This 2022 multi-country outbreak has mostly affected MSM. On January 5, 2023, a cumulative total of 83,943 confirmed cases have been reported to WHO from 110 countries in all 6 WHO regions, 96.6% being young men. To contain the spread of this outbreak, several countries have recommended vaccination with the 3rd generation live Modified Vaccinia Ankara (MVA), first as a post-exposure vaccination of exposed individuals, and rapidly after as a preventive pre-exposure strategy in at-risk individuals (multiple-partner MSM). In addition, WHO and the United States CDC issued specific risk-reduction guidelines targeted towards the MSM key population (including, but not limited to abstaining from risk exposure until two weeks after the second dose of the vaccine, avoiding kissing, limiting the number of sexual partners). As of January 2, 2023, only 8 of the 110 affected countries have reported an increase in the weekly number of cases, and 79/110 have not reported new cases for over 21 days, the maximum incubation period of the disease. The extent of contribution of vaccination and/or behavioral exposure mitigation strategies in this waning epidemic is unknown. Globally, vaccines are available in North America and Europe, whereas Africa remains without access to vaccines. For mpox, other countries in Africa will remain affected, which will be inequitable even if some countries in Europe and North America can control and eliminate mpox, as in other sexual reproductive health areas such a contraception, antenatal care and HIV PrEP. Programs to prevent mpox are being developed, and vaccines that this presentation will focus on, they will be discussed in the context of the entire prevention armamentarium including socio behavioral change, condoms, screening and treatment.

MPXV is a zoonosis, and its control will be critical to prevent future outbreaks worldwide. For mpox, discrimination and racism have been particularly directed against MSM, trans people, gender diverse communities and people from previously affected regions. This may prolong the disease outbreak by refraining people from coming forward for information or seeking testing or care, subsequently undermining public health efforts. Specific interventions addressing misconceptions and stigma are needed. Also, of particular interest are areas for rights-based approaches to address each level of this cascade in the context of client choice. It will be important to consider socio, economic and health system contexts for sustainability and scale up of the prevention of STIs. In this waning epidemic is unknown. Globally, vaccines are available in North America and Europe, whereas Africa remains without access to vaccines.
37 HOW THE HUSH COMPLEX PROTECTS YOUR GENOME FROM RNA-DERIVED RETROELEMENTS
Paul J. Lehner
Cambridge University, Cambridge, United Kingdom
Since the discovery of reverse transcriptase, retrotransposition, the reverse transcriptase-mediated conversion of RNA to cDNA and its subsequent genome integration is now recognized as the predominant route by which our genome acquires new genetic material. Indeed retroelements (retroviruses/retrotransposons) make up >40% of the human genome. This acquisition of new genetic material may be beneficial, increasing genome diversity and resilience, or potentially catastrophic as seen in HIV infection, exposing the genome to invasion from foreign, RNA-derived DNA. Retrotransposition is therefore tolerated, but needs to be closely regulated. My group discovered and characterised ‘HUSH’ (Human Silencing Hub), an epigenetic transcriptional repressor complex which silences invading DNA. HUSH defends the genome from retroelement attack from outside the cell i.e. retroviruses (including HIV) and from within the cell (LINE1 retrotransposons). HUSH is therefore a unique RNA-dependent genome surveillance system linking transcription to epigenetic gene silencing, HUSH’s ability to immediately identify and silence invading transgenes led to the question: What does HUSH recognize in the genome? We found that HUSH discriminates ‘self’ from ‘non-self’ genomic DNA by recognizing ‘intronless’ DNA, the essential hallmark of reverse transcription. Retroelements (retroviruses/retrotransposons) are RNA-derived and therefore lack non-coding introns. Intronless cDNA provides the ‘pathogen-associated molecular pattern’, which allows HUSH to distinguish invading retroelements from host genes. This discovery provides an elegant solution to how the host genome silences retroelement-derived invaders, provides the basis for genome immune-surveillance and defines a novel function for introns: to distinguish ‘self’ from ‘non-self’ DNA. The identification of an immune-surveillance system to protect the genome from ‘reverse genetic flow’ (from RNA to DNA) was unanticipated and reveals a new aspect of innate immunity - immune-surveillance of the genome, a compartment not thought to be accessible to the immune system. HUSH is not only of fundamental biological importance - HUSH inhibition also has major therapeutic potential. As HUSH represses long cellular CDNAS >1.5kb, strategies over the last 50 years to express genes for a wide range of purposes have, somewhat unwittingly, been a battle against HUSH. HUSH inhibition therefore has the potential to dramatically improve gene expression, and to release neo-antigens for cytotoxic T-lymphocyte recognition for immunotherapy.

38 THE SCIENCE OF AGING: LESSONS FOR HIV AT THE INTERFACE OF COMMONALITY AND HETEROGENEITY
George A. Kuchel
University of Connecticut, Farmington, CT, USA
In this presentation, Dr. George Kuchel will discuss the science of aging and potential lessons for HIV. He will describe our evolving understanding of the biology of aging and how heterogeneity involving these mechanisms may affect multi-morbidity, functional status, and frailty, as well as evidence for how HIV or its treatment may exacerbate these problems. The presentation will also outline opportunities for interventions on these pathways and considerations for trials testing interventions targeting biological aging that may affect multiple discrete disease outcomes and/or functional status.

39 STAGE THE SETTING: THE EPIDEMIOLOGY OF THE MPXV VIRUS
John Brooks
Centers for Disease Control and Prevention, Atlanta, GA, USA
This presentation will provide an epidemiologic snapshot of the multicity clade Iib 2022 mpox outbreak.

40 MOLECULAR PATHOGENESIS AND THERAPEUTIC TARGETS FOR MPXV VIRUS
Stuart N. Isaacs
University of Pennsylvania, Philadelphia, PA, USA
The 2022 global outbreak of mpox was unprecedented. It was remarkable in that there have been over 80,000 cases in locations that have not historically reported mpox disease. Also, rather than an initial zoonotic exposure, the outbreak has been driven by sustained human-to-human transmissions. The outbreak has tested our preparedness for a smallpox event and there is much we can learn about mpox and the effectiveness of the available countermeasures. Orthopoxviruses are large DNA viruses with genomes of ~200,000 base pairs and replication occurs in the cytoplasm of infected cells. During an infection, two forms of infectious virus are generated that have important roles in transmission and spread within a host. The outbreak has revealed the existence of a previously unidentified subclade (clade Iib) that likely has different properties than the other clades (clade I and Ila). Given the complex lifecycle requiring multiple viral enzymes and proteins involved in immune evasion, there are many therapeutic targets. Use of combination therapies in immunocompromised patients with mpox as well development of additional therapeutics are likely warranted to combat future outbreaks. To successfully contain the current outbreak, continued testing of patient populations with sexually transmitted infections will likely be needed. To prevent future global outbreaks, resources are needed in less wealthy countries.

41 IMMUNOLOGY AND VACCINOLOGY PERSPECTIVES FOR MPXV VIRUS
Sharon Frey
Saint Louis University, St Louis, MO, USA
This 20-minute presentation will include a brief history of mpox and the Modified Vaccinia Ankara (MVA) vaccination (JYNNEOS) approved for use against mpox in the US.

42 EFFECT OF NEW VIRAL VARIANTS ON NEUTRALIZING ANTIBODY POTENCY AND BREADTH
Penny L. Moore
University of the Witwatersrand, Johannesburg, South Africa
South Africa has experienced several waves of SARS-CoV-2 infection following a slow vaccine roll-out. This resulted in extremely high population infection, with >98% of South Africans seropositive, and multiple cases of reinfection with diverse variants, which has shaped the quality and titers of antibody responses. South Africa also bears the brunt of the HIV pandemic, with 7.5 million PLWH. Of these, 2 million people do not access antiretroviral therapy, with implications for their ability to mount effective humoral responses, and to clear SARS-CoV-2 infection. Here, Moore will describe population-level vaccine-induced and hybrid humoral immunity in PLWH and HIV-uninfected individuals.

43 MEMORY B CELL RESPONSES TO NEW VIRUS VARIANTS: RELEVANCE TO THE CONCEPT OF ORIGINAL A
Marion Pepper
University of Washington, Seattle, WA, USA
In this presentation, Dr. Marion Pepper will discuss Memory B cell responses to COVID-19.

44 EPITOPE MASKING DURING A RECALL RESPONSE: IMPLICATIONS FOR COVID AND HIV VACCINES
Facundo Batista
Harvard University, Cambridge, MA, USA
Some of the first immunological experiments of the 20th century—on antibody feedback inhibition—demonstrated that past exposure does not merely expedite the humoral response to secondary challenges but, rather, alters the nature of that response. Despite this history, the mechanisms underpinning these classic observations and their relationship to modern vaccine design remain obscure. Circulating antibodies produced during a primary challenge can have enhancing or, conversely, inhibitory effects on later humoral responses. Using preclinical vaccine models, we dissected this apparent contradiction to find that the interaction between serum antibodies and the entry of cognate naive B cell lineages to germinal centers was determined by the interplay of breadth, affinity, and titers. Epitope-focused vaccine designs for HIV-1 elicit circulating antibodies capable of entirely obstructing their cognate naive B cells, with important implications for the ongoing design of boost-phase immunogens. Conversely, SARS-CoV-1 receptor binding domain (RBD) immunization elicits a polyclonal serum response that enhances the proportion of high-affinity cognate B cells in germinal centers, providing a partial explanation for the effectiveness of current boost protocols but also presaging diminishing returns unless new epitopes are introduced. The resolution of these surface-level contradictions points toward a unified interactive model and emphasizes that, to move vaccinology forward, the basic biology of the humoral immune system cannot remain a black box.
CASE PRESENTATIONS, DISCUSSION, AND ANSWERS TO AUDIENCE QUESTIONS

Georg Behrens1, Vidya Mave2, Judith S. Currier3, Jennifer Jao4, Peter Reiss5, Pam S. Douglas6, Ntobeko Ntsu7, Donal O’Shea8, Giada Sebastiani9
1Medizinische Hochschule Hannover, Hannover, Germany; 2Center for Infectious Diseases, Pune, India; 3University of California Los Angeles, Los Angeles, CA, USA; 4Northwestern University, Chicago, IL, USA; 5University of Amsterdam, Amsterdam, Netherlands; 6Duke University School of Medicine, Durham, NC, USA; 7University of Cape Town, Cape Town, South Africa; 8University College Dublin, Dublin, Ireland; 9McGill University, Montreal, QC, Canada

This case-based and interactive session will provide an up-to-date overview of the pathogenesis, prevention, and treatment of cardiovascular disease and heart failure in HIV medicine. Experts will discuss the state-of-the-art management of non-alcoholic fatty liver disease (NAFLD), including its clinical indicators in people living with HIV. The case presentations and panel discussions will reveal the emerging science on the tight regulation of adult body weight and how obesity affects the immune system in a way that is not dissimilar to the impact of HIV. We will provide insights into new treatments transforming the obesity landscape. After attending this session, the delegates will have an advanced understanding of the development, prevention, and treatment of the most important cardio-metabolic complications in HIV medicine and the consequences for clinical management.

COUNTERING VACCINE AND HEALTH MISAND DISINFORMATION: AN EVIDENCE-BASED APPROACH

Scott C. Ratzan
City University of New York, Princeton, NJ, USA

COVID-19 was the first pandemic that unfolded in an information environment transformed by the ubiquitous mass and social media. Sensational misinformation and deliberate disinformation proliferated capturing hearts and minds. Traditional medical and public health organization’s voices were well-intentioned and informed to communicate about the threat of SARS-CoV-2 infection and appropriate countermeasures (for example, masking and vaccines) but were overwhelmed. This new reality on communicating health and science was called out by the World Health Organization in 2020 as an Infodemic. In 2021, the U.S. Surgeon General issued his first report entitled “Confronting Health Misinformation: The U.S. Surgeon General’s Advisory on Building a Healthy Information Environment” urging at scale investment to tackle misinformation.

Yet, while warnings were issued, mis- and disinformation proliferated with a palpable casualty emerging with waning vaccine confidence and uptake globally. This fundamental tenet of public health—vaccination—is at risk as a credible defense against disease and illness. This is not due to the microbial vectors but instead the societal response gathering momentum during the COVID “infodemic”.

This “endemic” challenge on how we communicate health and science will be described with an evidence-based perspective vetted in the field of communication with conceptual, scientific and theoretical grounding. This presentation will offer approaches to help build scientific, health and vaccine literacy, counter mis- and dis-information and foster strategic health communication capabilities at all levels of society.

WHAT CAN I DO? THE CHALLENGE OF HEALTH MISINFORMATION ONLINE

Emily Vraga
University of Minnesota, Minneapolis, MN, USA

Concerns about misinformation are not new, but the rise of social media has supercharged the risks of misinformation to the populace, especially when that misinformation deals with vaccination and communicable disease. Studies have documented the prevalence of online misinformation on a range of topics and platforms. Social media features like fierce competition for audience attention, the potential for online echo chambers, and the prominent role of influencers explain the success of online misinformation. Journalism and health organizations contribute to the problem when they do not follow best practices for communicating scientific consensus and addressing uncertainty in health recommendations. However, understanding the impact of such misinformation is further complicated by recognizing that prevalence of misinformation does not equate to exposure.

As a result, scholars and practitioners have turned their attention to identifying best practices for addressing misinformation and reducing its spread. Three strategies for responding to the challenge of misinformation hold the most promise. First, prebunking misinformation involves giving people the tools to build resilience against misinformation before it spreads. This can happen by inoculating people against common misinformation strategies, educating the public with news and health literacy skills, and rebuilding trust in expert institutions tasked with conveying accurate information to the public. Second, debunking misinformation requires directly responding to and offering corrective information to concrete cases of viral misinformation. Third, promoting high quality information ensures that the public can easily find and identify the information they need, filling potential information voids and avoiding overload. Such high quality information must come from a variety of trusted sources to ensure its success in reaching the diverse audiences. Given the scope and speed with which misinformation can spread on social media, no one strategy will be sufficient to mitigate its potential harms to the public. Instead, we must build layers of resilience to misinformation by promoting high quality information, prebunking misinformation when it is likely to spread, and debunking misinformation after it has gone viral. This response requires concerted efforts from experts, journalists, platforms, and users alike, and each has a role to play.

WHAT’S FUELING THE SPREAD OF MISINFORMATION: LOOKING BEYOND FAKE FACTS

Heidi J. Larson
London School of Hygiene & Tropical Medicine, Brussels, Belgium

Misinformation is a systemic issue that breeds on distrust. The solution lies in not only informational debunking, but trust building to counter the fertile ground factors that fuels the spread of viral misinformation. This presentation, “What’s fueling the spread of misinformation: Looking beyond fake facts” will focus on the historic and current triggers and fertile group factors that fuel the contagion of mis- and disinformation with impacts of human health and risk disruption to clinical trials and health interventions. Multiple examples and approaches to mitigate the spread of misinformation will be presented.
100 LENACAPAVIR INHIBITS VIRION MATURATION BY BLOCKING FORMATION OF CAPSID PENTAMERS
Szu-Wei Huang, Lorenzo Briganti, Guochao Wei, Arun Annamalai, Stephanie Bester, Nikoloz Shkribal, Mamuka Kvaratskhelia
University of Colorado, Aurora, CO, USA

**Background:** HIV-1 capsid contains ~1,500 capsid proteins (CA) arranged into ~250 hexamers and exactly 12 pentamers, which introduce curvatures to allow assembly of a closed, conical structure during virion maturation. Because CA contributes to different aspects of the viral replication cycle, small molecules that target this protein have the potential to exert their effects in both early and late stages of the virus life cycle. Lenacapavir (LEN) is a first-in-class, long-acting, and ultra-potent HIV-1 CA inhibitor. We and others have previously elucidated a multi-modal mechanism of action of LEN during early steps of infection. LEN also potently inhibits late steps of HIV-1 replication. However, the underlying mechanism for this antiviral activity is unclear. In clinical trials resistance mutations to LEN emerged in 8 participants (6 with M66I). However, the underlying mechanism for this major drug resistant substitution is unclear.

**Methods:** We used transmission electron microscopy to monitor LEN effects on assembly of capsid-like particles in vitro, high-resolution X-ray crystallography to investigate effects of the M66I change on LEN and CPSF6 binding to CA hexamers, and virology assays to delineate LEN effects during late steps of HIV-1 replication.

**Results:** Unexpectedly, we found that during assembly of capsid-like particles in vitro, LEN specifically blocked formation of CA pentamers but not hexamers. Consequently, unlike cellular cofactor IP6, which promotes formation of conical, closed mature structures comprised of both hexamers and pentamers, LEN treatments yielded open-ended tubular assemblies, which contained exclusively hexamers. Consistent with these observations, our virology assays revealed opposing effects of IP6 and LEN during virion maturation. Additionally, we have elucidated the structural basis for M66I mediated viral resistance to LEN. Remarkably, the beta-side change of Ile66 introduced steric hindrance specifically with respect to LEN, whereas the M66I change had no effects on IP6 mediated assembly of correctly matured virions or capsid binding to CPSF6, which engages the same hydrophobic CA pocket as LEN.

**Conclusion:** Our studies have revealed the previously undescribed antiviral mechanism of action of and viral resistance to LEN. These findings provide means for developing second generation CA inhibitors with a higher barrier to resistance.

101 INHIBITION OF NEUTRAL SPHINGOMYELINASES 2 BLOCKS HIV-1 MATURATION
Abdul Waheed, Yanan Zhu, Seung-Wan Yoo, Ferri Soheilian, Eva Agostino, Lwar Naing, Pragney Deme, Yun Song, Peijun Zhang, Barbara S. Slusher, Norman Haughey, Eric O. Freed

National Cancer Institute, Frederick, MD, USA; University of Oxford, Oxford, United Kingdom; Johns Hopkins University School of Medicine, Baltimore, MD, USA; Diamond Light Source, Oxford, UK.

**Background:** Sphingomyelinases (SMases) are key enzymes that hydrolyze sphingomyelin (SM) to generate phosphorylcholine and ceramide. Ceramides at the plasma membrane (PM) are critical for a variety of biological functions, including the formation and stabilization of lipid rafts that are assembly sites for HIV. Neutral sphingomyelinase 2 (nSMase2) is the major sphingomylinase in mammalian cells that generates ceramide at the PM. Previous studies identified phenyl(R)-(1-3-((3,4-dimethoxyphenyl)-2,6-dimethylimidazol[1,2-b]pyridazin-8-yl)pyrrolidin-3-yl)carbamate (PDDC) as a potent and selective inhibitor of nSMase2. Because of its key role in ceramide biosynthesis, we hypothesized that nSMase2 might be required for HIV-1 assembly, release, or maturation.

**Methods:** To investigate the role of nSMase2 in HIV-1 replication, we either knocked down nSMase2 with siRNA or treated virus-producing cells with PDDC and monitored virus assembly, release, maturation, and infectivity by using a variety of techniques. In vitro selection experiments were performed to obtain PDDC-resistant virus. The impact of PDDC on virus release and morphology of other retroviruses was also analyzed.

**Results:** We found that the nSMase2 inhibitor PDDC, or shRNA-mediated depletion of nSMase2, impairs HIV-1 Gag and GagPol processing, resulting in a profound impairment in particle maturation and infectivity. The defect in Gag and GagPol processing is not due to direct inhibition of HIV-1 protease activity or reduced incorporation of GagPol into virions. Analysis by thin section transmission electron microscopy and cryo-electron tomography shows that disrupting nSMase2 in virus-producer cells alters the morphology of HIV-1 particles and prevents particle maturation. We found that disruption of nSMase2 blocks the maturation of HIV-1, SIVmac, HIV-2, and equine infectious anaemia virus but not that of murine leukemia virus or feline immunodeficiency virus. Using a sub-optimal dose of PDDC in HIV-1-infected human T cells we were able to select and identify mutations in Gag that confer partial resistance to PDDC.

**Conclusion:** These studies demonstrate a role for nSMase2 in lentiviral morphogenesis and maturation and suggest that specific alterations in the lipid composition of assembling virus particles profoundly impact late stages of the lentiviral replication cycle.

102 INVESTIGATING THE ROLE OF CAPSID STABILITY IN INNATE IMMUNE SENSING OF HIV-1 CORES
Jenna E. Eschbach, Sebba B. Kutluay
Washington University in St. Louis, St. Louis, MO, USA

**Background:** The emergence of antiretroviral drug resistance has hindered progress toward combatting the ongoing HIV/AIDS epidemic. A promising drug target, HIV-1 capsid (CA) is a genetically fragile protein that plays essential roles throughout the viral replication cycle. We recently demonstrated that destabilization of the CA lattice increases the propensity to form aberrant virus particles. In these particles, the genomic RNA (gRNA) and enzymes are localized between the CA lattice and viral envelope. We showed that the lack of protection by the CA lattice results in premature loss of gRNA and integrase (IN) in a proteasome-independent manner. Recent studies have implicated that CA may shield viral nucleic acids from the host sensor proteins that initiate antiviral responses. Here, we directly tested whether tampering with the stability of the HIV-1 CA lattice results in premature exposure and innate sensing of viral nucleic acids in infected cells.

**Methods:** To this end, we examined the expression levels of interferon stimulated genes (ISGs) upon infection of monocyte-like THP-1 cells with WT and mutant viruses bearing cores with altered stability. THP-1 cells were infected with equivalent numbers of VSV-G pseudotyped viruses with CA stabilizing or destabilizing mutations. ISG induction was assessed by qRT-PCR at 24 and 48 hours post-infection. We applied one-way or two-way ANOVAs to continuous variables and assessed statistical significance at the 0.05 level.

**Results:** The WT virus and viruses with destabilizing mutations induced low to moderate levels of ISG expression in a reverse transcription-dependent manner. Contrary to our predictions, we found that stabilization of the capsid lattice through the E45A substitution induced a more potent innate immune response. Sensing of viruses bearing hyperstable capsids was also dependent on reverse transcription and the GAS-STAT3 pathway. Interestingly, we found a differential requirement for TET1 exonuclease for sensing of WT versus CA E45A HIV-1 and no involvement of the recently reported CA-binding protein PBP1 in innate sensing.

**Conclusion:** Taken together, our study provides novel insight into yet another proposed role of CA in the evasion of innate immune sensing and suggests that viral nucleic acids remain accessible to innate sensing despite capsid stabilization.

103 MUTATIONS OUTSIDE INTEGRASE LEAD TO HIGH-LEVEL RESISTANCE TO DOLUTEGRAVIR
Yuta Hikichi, Jennifer L. Groebner, Ann Wiegand, John W. Mellors, Mary F. Kearney, Eric O. Freed

National Cancer Institute, Frederick, MD, USA; University of Pittsburgh, Pittsburgh, PA, USA.

**Background:** We reported that mutations in the envelope glycoprotein (Env) can broadly reduce HIV-1 susceptibility to ARVs. The aim of the current study was to examine the pathway(s) by which HIV-1 develops high-level resistance to the integrase (IN) strand transfer inhibitor ( INSTI) dolutegavir (DTG).
Methods: Long-term passaging of lab-adapted and primary viral isolates using the SupT1 T-cell line was performed over nearly one year with an escalating concentration (0.1 – 2,000 nM) of DTG. Viral sequence analysis was performed longitudinally. Identified mutations were introduced into WT HIV-1 molecular clones and the replication kinetics and viral infection through cell-cell contact were examined in the presence and absence of drug. To measure the multiplicity of infection (MOI), we monitored viral replication by co-infection with eGFP- and mRuby-expressing reporter viruses harboring the Env mutations.

Results: In a manner independent of viral isolate and coreceptor usage, HIV-1 became resistant to DTG by sequentially acquiring mutations in Env and Gag-nucleocapsid (NC) in the absence of resistance mutations in IN. The selected NC mutations clustered in the zinc-finger domain and conferred modest (3-5 fold) resistance to αNzTs. An Env mutant, 7XEnv, containing seven substitutions (V85A, S162K, R289K, Q363R, A541V, V693I and G825E) exhibited faster-than-WT replication and resistance to multiple classes of ARVs, with the fold resistance being markedly higher for αNzTs. Viral transmission of 7XEnv through cell-cell contact is 15-fold more efficient than WT, resulting in a higher MOI and reduced sensitivity to DTG. Viral infection using VSV-G-pseudotyped viruses over a range of MOIs revealed that αNzTs are more readily overgrown by high MOI compared to RT inhibitors. Co-infection experiments using fluorescently tagged reporter viruses demonstrated that 7XEnv infection leads to a higher number of cells expressing multiple proviruses compared to WT.

Conclusion: These findings demonstrate that a combination of mutations in Env and NC can confer high-level resistance to αNzTs in the absence of IN mutations. The Env mutations overcome inhibition by αNzTs through increased MOI mediated by highly efficient cell-cell transfer. These results advance the understanding of how HIV-1 can evolve resistance to ARVs in the absence of mutations in drug-target genes and provide new insights into the contribution of cell-cell transfer to viral replication and drug resistance.

104 MX2 ANTIVIRAL SPECIFICITY IS AFFECTED BY GTPase ACTIVITY AND CAPSID-CypA INTERACTIONS
Bailey Layish, Haley Flick, Ram Goli, Mariah Cashbaugh, Robert Z. Zhang, Melissa Kane
University of Pittsburgh, Pittsburgh, PA, USA

Background: MX2 (myxovirus resistance 2), an antiviral protein whose expression is strongly upregulated by type 1 interferon, localizes to the nuclear pore complex (NPC) and inhibits HIV-1 infection by preventing nuclear import of the viral preintegration complex. The HIV-1 capsid (CA) is the major viral determinant for sensitivity to MX2, and complex interactions between MX2, CA, nucleoporins (Nups), cyclolinophin A (CypA), and other cellular proteins influence the outcome of viral infection. Like MxA, MX2 is comprised of a GTPase domain connected to a carboxy-terminal stalk domain via a tripartite bundle-signaling element (BSE), however while GTPase function and higher order oligomerization are generally required for the antiviral activity of MxA, they are dispensable for the anti-lentiviral activity of MX2.

Methods: To explore the interactions between MX2, the viral CA, and CypA, we utilized a CRISPR/Cas9 approach to generate CA-GFP knock-out cell lines as well as cells that express CypA from its endogenous locus, but with specific point mutations that would abrogate CA binding but should not affect enzymatic activity or cellular function.

Results: We found that antiviral activity of MX2 was altered in CypA mutant cell lines in a virus-specific manner, as elimination of CA-CypA interactions reduced the antiviral activity of MX2 against HIV-1 but not HIV-2. We additionally found that infection of CA-GFP knock-out and point mutant cell lines with wild-type HIV-1 and CA mutants recapitulated the phenotypes observed upon cyclosporine A (CsA) addition, indicating that effects of CsA treatment are the direct result of blocking CA-CypA interactions and are therefore independent from potential interactions between CypA and MX2 or other cellular proteins. Interestingly, abrogation of GTP hydrolysis by MX2 conferred enhanced antiviral activity when CA-CypA interactions were abolished. Elimination of GTPase activity also altered the Nup requirements for MX2 localization and activity against wild-type HIV-1 and the G89V CA mutant which does not bind CypA.

Conclusion: Our data demonstrate that the antiviral activity of MX2 is affected by CypA-CA interactions in a virus-specific and GTPase activity-dependent manner. These findings further highlight the importance of the GTPase domain of MX2 in regulation of substrate specificity and interaction with nucleocytoplasmic trafficking pathways.
HUMAN ANTI-ACE2 MONOCLONAL ANTIBODIES AS PAN-SARBEVIRUS PROPHYLACTIC AGENTS

Fengwen Zhang, Jesse Jenkins, Renan de Carvalho, Sandra Nakandakari-Higa, Teresia Chen, Morgan Abernathy, Elisabeth Nyakatura, Ivo Lorenz, H.-Heinrich Hoffmann, Charles Rice, Gabriel Victora, Christopher Barnes, Theodora Hatziioannou, Paul D. Bieniasz

Rockefeller University, New York, NY, USA, Stanford University, Stanford, CA, USA, Tri-I TDI, New York, NY, USA

Background: Human monoclonal antibodies from convalescent individuals that target the SARS-CoV-2 spike protein have been deployed as therapeutics against SARS-CoV-2. However, nearly all of these antibodies have been rendered obsolete by SARS-CoV-2 variants that evolved to resist similar, naturally occurring antibodies. Moreover, Most SARS-CoV-2-specific antibodies are inactive against divergent sarbecoviruses.

Methods: By immunizing mice that carry human immunoglobulin variable gene segments we generated a suite of fully human monoclonal antibodies that bind the human ACE2 receptor (hACE2) rather than the viral spike protein and were engineered to lack effector functions such as ADCC.

Results: These ACE2 binding antibodies block infection by all hACE2 binding viruses that might emerge as future pandemic threats. By immunizing mice that carry human immunoglobulin variable gene segments we generated a suite of fully human monoclonal antibodies that bind the human ACE2 receptor (hACE2) rather than the viral spike protein and were engineered to lack effector functions such as ADCC.

Conclusions: ACE2 binding antibodies should be useful prophylactic and treatment agents against any current and future SARS-CoV-2 variants, as well as hACE2-binding sarbecoviruses that might emerge as future pandemic threats.

SARS-CoV-2 VARIANTS EVOLVED CONVERGENT STRATEGIES TO REMODEL THE HOST RESPONSE


Quantitative Biosciences Institute Coronavirus Research Group (QCRG)

University of California Los Angeles, Los Angeles, CA, USA, University College London, London, United Kingdom, University of California San Francisco, San Francisco, CA, USA, Texas Biomedical Research Institute, San Antonio, TX, USA, John Hopkins School of Medicine at Mt Sinai, New York, NY, USA

Background: Five variants of concern (VOCs) have dominated COVID-19 disease etiology since 2020—Alpha, Beta, Gamma, and Omicron—possessing over 125 defining genetic alterations. Here, we used global proteomic and genomics approaches to study the host responses and selective forces driving VOC evolution.

Methods: We infected Calu-3 human lung epithelial cells with 5 VOCs and 2 wave 1 (W1) controls and performed mass spectrometry abundance proteomics, phosphoproteomics, and mRNA sequencing at 10 and 24 hours post infection. We additionally performed affinity purification mass spectrometry (APMS) by individually expressing all VOC mutant viral proteins (S2) and corresponding W1 forms in human cells to quantify differential virus-host protein-protein interactions. Data was integrated using network modeling and bioinformatics to pinpoint VOC-specific differences. Four novel mutant viruses were developed using reverse genetics technology to validate the impact of specific genetic alterations.

Results: We discovered VOCs evolved divergent molecular strategies to remodel the host response by modulating viral RNA and protein levels (most notably of N, Orf9b, and Orf6), altering nucleocapsid phosphorylation, and rewiring virus-host protein complexes. Integrative systems analyses revealed that Alpha, Beta, Gamma, and Delta ultimately converged in the suppression of interferon stimulated genes (ISGs) relative to W1 viruses, but Omicron BA.1 did not, and Delta induced more pro-inflammatory genes compared to other VOCs. Altered regulation of ISGs correlated with the expression of viral innate immune antagonist proteins, including Orf6, N, and Orf9b; for example, Omicron BA.1 depicted a 2-fold decrease in Orf6 expression. We identified mutations that altered expression of Orf9b (N D3L and N -3A del) and the novel VOC protein N* (N R203K/G204R), and confirmed Orf6 innate immune antagonism using recombinant virus technology. Remarkably, Omicron BA.4 and BA.5 regained enhanced human-to-human transmission.
110 STANDARD VS DOUBLE DOSE DOLUTEGRAVIR IN HIV-ASSOCIATED TUBERCULOSIS: WEEK 48 RESULTS
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1University of Cape Town, Cape Town, South Africa, 2University of Liverpool, Liverpool, United Kingdom
Background: Rifampininduces genes important in the metabolism and transport of dolutegravir. The resulting drug-drug interaction can be overcome by doubling the daily dose of dolutegravir, but this increases costs and risk of stockouts in high burden settings. We recently presented week 24 findings from the RADIANT-TB trial that showed adequate virological suppression with both standard and double dose dolutegravir in participants on rifampicin-based antiretroviral therapy. Here we present week 48 outcomes in order to detect emergence of dolutegravir resistance, and adherence assessed by tenofovir-diphosphate (TFV-DP) on dried blood spots (DBS).

Methods: SimplifiTB study was an open-label safety and efficacy study in 303 DS-TB patients randomized 1:1 to 4-months BPaMZ vs 6-months HRZE, and 6-months BPaMZ in 152 DR-TB patients. Primary efficacy endpoint was time to culture negative status through 8 weeks; a key secondary endpoint was relapse-free cure at week 52. In a separate analysis, 3 groups representing PaZ-containing regimens (PaZX, n=841), BPaL (n=290), and HRZE (n=340) were aggregated across all 6 TB Alliance studies of ≥8 weeks. Liver safety (max treatment-emergent (TE) ALT elevations) was analyzed through 8 weeks.

Results: In the SimplifiTB MITT population, 47% and 84% of DS-TB participants (and 86% of DR-TB participants) were culture-negative by week 8 in HRZE and 4BPaMZ arms respectively, meeting superiority of 4BPaMZ over HRZE: HR 2.9 (95% CI 2.2 – 4.0) (figure 1). At week 52, 7%, 17%, and 17% of participants had unfavourable outcomes in the HRZE, 4BPaMZ and 6BPaMZ arms, respectively (TB-MITT population); 4-month BPaMZ did not meet non-inferiority (12% margin) vs HRZE. The higher proportion of BPaMZ unfavourable outcomes was driven primarily by hepatotoxicity-related treatment discontinuations: HRZE (0%), 4BPaMZ (7%) and 6BPaMZ (6%). In the PP population (treatment discontinuations excluded), 4BPaMZ was non-inferior to HRZE: risk difference 1.5% (95% CI: 2.6%–0.6%). The multidrug analysis showed incidences (95% CI) of TE ALT elevations >3ULN of 10.8% (8.8–13.1), 8.6% (5.7–12.5), and 5.6% (3.4–8.6), respectively, in those receiving PaZX, BPaL, and HRZE. Severe (grade 4) TE ALT elevations >8ULN occurred in 4.6% (3.3–6.3), 1.0% (0.2–3.0), and 2.7% (1.2–5.0), with median increases of 20x, 10x, and 10x, respectively, for PaZX, BPaL, and HRZE.

Conclusion: SimplifiTB results validate observations from pre-clinical relapsing mouse model experiments which identified BPaMZ as a regimen with high efficacy and treatment shortening potential, but hepatotoxicity precluded treatment completion in ~6–7% of patients. The multidrug analysis showed that PaZ-containing regimens were associated with a hepatic safety profile distinct from BPaL, with a higher incidence and degree of grade 4 ALT elevations. SimplifiTB Primary efficacy analysis Time to culture negative status by weeks 48 (MITT)

111 INCREASED TB PREVENTIVE THERAPY COVERAGE WITH INTEGRATED COMMUNITY-BASED IPT AND ART
Adrienne E. Shapiro1, Adam Szpiro1, Kombi Sausi2, Nsika Sithole3, Olivier Koole4, Meighan Krows1, Torin Schaafsma5, Maryam Shahmanesh6, Heidi van Rooyen7, Connie Celum1, Alastair van Heerden1, Ruanne Barnabas3
DO ART Study Team
University of Washington, Seattle, WA, USA, 1Human Sciences Research Council, Cape Town, South Africa, 2Africa Health Research Institute, Durban, South Africa, 3ICAP at Columbia University, Maputo, Mozambique, 4Human Sciences Research Council, Sweetwaters, South Africa, 5Massachusetts General Hospital, Boston, WA, USA

Background: Tuberculosis (TB) is a leading cause of mortality among people living with HIV and is prevented by isoniazid preventive therapy (IPT). Uptake is low despite guidelines that recommend IPT for all people with HIV (PWH) in high TB prevalence settings, in part due to limited integration into ART services. We tested the efficacy of integrated community-based ART and IPT services on IPT initiation and continuation.

Methods: The DO ART Study tested integrated IPT and community-based ART in KwaZulu-Natal, South Africa. PWH were randomized 1:1:1 to 1) clinic-based services including standard-of-care IPT; 2) community-based ART and IPT initiation, delivery, quarterly refills, and decentralized monitoring; or 3) hybrid services with clinic ART and IPT initiation and community follow-up. In intervention groups, participants were screened by lay health workers for TB symptoms and contraindications to IPT, and if eligible, were offered IPT 1 month after ART initiation. Quarterly IPT refills were synchronized with ART dispensing. IPT initiation and adherence were assessed through chart review, medication dispensing logs, and quarterly self-report. IPT continuation was defined as refill receipt. A subset of participants had urine tested for isoniazid metabolites at community IPT refill visits to assess concordance with self-reported adherence.

Results: Between 2/2017-3/2019, 1,212 PWH, including 540 (45%) men, were randomized. Of 1,039 (441, 42% men) who initiated ART, 573 (55%) initiated IPT during 12-months follow-up. Compared to clinic-based IPT uptake of 20%, IPT initiation was 90% in the community-based delivery group (RR=4.59, 95% CI 3.69-5.71), and 48% in the hybrid group (RR=2.44, 95% CI 1.91-3.12). Among participants initiating IPT, continuation was similar in the hybrid (84%) and community-based delivery (89%) groups (RR=1.73, 95% CI 1.33-2.34); compared to 48% continuation in the clinic-based group. Gender was not associated with IPT use. Isoniazid metabolites were present in 164/259 (63%) of participants who reported taking IPT.

Conclusion: Community-based delivery of IPT with ART was associated with more than four-fold higher rate of IPT initiation and two-fold higher
continuation than standard clinic-based care in South Africa. High levels of IPT uptake and continuation were achieved through integration into community-based ART delivery, and demonstrated feasibility, adherence, and acceptability. Urine testing can complement self-reported adherence measures.

IPT initiation and adherence cascade in DO ART

![Graph showing IPT initiation and adherence cascade in DO ART]

**RCT OF ECONOMIC INCENTIVES FOR RECOMMENDED ALCOHOL USE AND INH ADHERENCE AMONG PWH**

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 Universidad de California San Francisco, San Francisco, CA, USA, 2Mbarara University of Science and Technology, Mbarara, Uganda, Infectious Diseases Research Collaboration, Mbarara, Uganda, 3Boston University, Boston, MA, USA, 4University of Pennsylvania, Philadelphia, PA, USA, 5Washington State University, Spokane, WA, USA, 6National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA, 7University of Pennsylvania, Philadelphia, PA, USA

**Background:** Alcohol use is common among persons with HIV (PWH) and is a risk factor for TB disease, non-adherence to INH preventive therapy (IPT) and antiretroviral therapy (ART), and hepatotoxicity during IPT. Interventions are urgently needed to reduce hazardous alcohol use and improve IPT adherence among PWH.

**Methods:** We conducted a 2x2 factorial randomized controlled trial among PWH (≥18 years) on ART, with latent TB infection (PTD≥5mm) and hazardous alcohol use in Uganda. We randomized 680 participants (1:1:1 initiating 6-months of IPT: no incentives (Arm 1 control) or incentives contingent on no recent alcohol use (Arm 2), recent INH adherence (Arm 3), or both (awarded independently, Arm 4). The escalating financial incentives were contingent on monthly point-of-care (POC) urine tests that were negative for ethyl glucuronide, a biomarker of recent alcohol use (Arm 2 & 4), or positive on IsoScreen, a biomarker of recent INH use (Arms 3 & 4). The primary alcohol use outcome was non-hazardous use by self-report (Alcohol Use Disorder Identification Test-Consumption [AUDIT-C< 3 women, < 4 men], prior 3 months) and secondary endpoints in a randomized controlled trial of economic incentives for reduced alcohol use and/or INH adherence by monthly POC testing led to significant reductions in biomarker-confirmed alcohol use, but no change in INH adherence among PWH with latent TB infection and hazardous alcohol use receiving IPT. This trial is among the first to show efficacy of incentives in reducing alcohol use in sub-Saharan Africa.

**Primary and secondary endpoints in a randomized controlled trial of economic incentives for reduced alcohol use and increased INH adherence among PWH with latent TB infection and hazardous alcohol use in Uganda.**

### 113 EFFICACY AND SAFETY OF 8-WK TUBERCULOSIS TREATMENT REGIMENS IN THE TRUNCATE-TB TRIAL

**Nicholas Paton**, Christopher Cousins, Qingshu Lu, Rajesh Moorakonda, Celina Suresh, Erilna Burhan, Vincent Balanag, Christine Sekgagga-Wiltshire, Ancheale Avhingsanon, Rohit Sarin, Angela Crook

The TRUNCATE-TB trial team

National University of Singapore, Singapore, Singapore, 2National University of Singapore, Singapore, 3Cerri, Singapore, Singapore, 4Cerri, Singapore, 5Peninsula Hospital, Jakarta, Jakarta, Indonesia, 6Centre for Disease Control, Philippines, 7Quezon City, Philippines, 8Infectious Diseases Institute, Kampala, Uganda, 9Red Cross AIDS Research Center, Bangkok, Thailand, 10National Institute of TB and Respiratory Diseases, Delhi, India, 11Medical Research Council, London, United Kingdom

**Background:** The TRUNCATE-TB trial (NCT03474198) found that the TRUNCATE strategy – initial treatment for 8 weeks (with BDQ/LZD regimen; extended up to 12 weeks for unsatisfactory clinical response); post-treatment follow-up; prompt retreatment (standard regimen) for relapse – was non-inferior to the standard treatment strategy at 96 weeks. Here we analyze efficacy and safety of the trial regimens (distinct from the efficacy and safety of the overall TRUNCATE strategy in which they were deployed).

**Methods:** Participants with rifampin-susceptible pulmonary TB at 18 Asian African sites were randomized (adaptive design) to receive the standard regimen for 24 weeks or one of four novel 5-drug regimens for 8-weeks (up to 12 weeks; table). We assessed regimen efficacy using unfavorable outcome (UFO; composite including treatment failure; relapse; death; non-attendance at week 96, without evidence of prior disease clearance); and estimated Bayesian probability that UFO risk difference vs standard treatment was < 12%. Safety was assessed by adverse events of grade ≥3 (AE≥3) during/within 30 days of stopping the initial regimen.

**Results:** Of the 674 randomized participants (male 62%, mean age 74%, lung cavitation 54%), 4 withdrew/were lost to follow-up by week 96; overall, 80% assigned novel regimens stopped after exactly 8 weeks, 9% extended up to 10 weeks; 3% extended up to 12 weeks. Three arms (standard, hRIF/LZD, BDQ/LZD) enrolled to full sample size; two (hRIF/CFZ, RPT/LZD) ceased enrollment early for pragmatic reasons (not efficacy/safety; n in Table). Proportions with UFO were 3.9%, 25.0% and 13.8% in the standard, hRIF/LZD and BDQ/LZD arms respectively; probability that risk difference vs standard arm was < 12% was 0.01 in hRIF/LZD and 0.81 in BDQ/LZD arm; the risk difference vs standard arm was reduced in subgroups with lower disease burden. Proportions with AE≥3 were 13.8%, 10.9%, and 11.1% in the standard, hRIF/LZD and BDQ/LZD arms respectively; 2 (1.1%) acquired BDQ resistance in the BDQ/LZD arm, none acquired resistance in other arms.

**Conclusion:** Unfavorable outcome was more frequent with 8-week regimens than the 24-week regimen, as expected. However, with the BDQ/LZD regimen the excess was modest and likely can be reduced further by adjusting criteria for treatment extension by subgroup; the regimen was safe. An 8-week initial treatment duration appears to be a feasible target for most people with TB, with the excess of unfavorable outcomes manageable within the TRUNCATE strategy.

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### Table

<table>
<thead>
<tr>
<th>Arm</th>
<th>Intervention</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hRIF/LZD</td>
<td>17.4% (5.9)/6.3%</td>
<td>6.5% (0.3)/0.5%</td>
<td>0.003</td>
</tr>
<tr>
<td>BDQ/LZD</td>
<td>7.2% (2.9)/7.2%</td>
<td>7.2% (2.7)/7.2%</td>
<td>0.944</td>
</tr>
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</table>

*Note: UNA=Unfavorable outcome, including TB treatment failure, relapse, death, non-attendance at week 96 with evidence of prior disease clearance.*
Table: Randomised drug regimens

<table>
<thead>
<tr>
<th>Arm (n randomised)</th>
<th>Drugs in regimens</th>
<th>Arm 1 (n randomised)</th>
<th>Drugs in regimens</th>
<th>Arm 2 (n randomised)</th>
<th>Drugs in regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard regimen, 24w</td>
<td>Lamivudin 150mg</td>
<td></td>
<td>Lamivudin 150mg</td>
<td></td>
<td>Lamivudin 150mg</td>
</tr>
<tr>
<td>High-dose lamivudin and tenofovir, 24w</td>
<td>Lamivudin 150mg, Tenofovir</td>
<td></td>
<td>Lamivudin 150mg, Tenofovir</td>
<td></td>
<td>Lamivudin 150mg, Tenofovir</td>
</tr>
<tr>
<td>High-dose lamivudin and efavirenz, 24w</td>
<td>Lamivudin 150mg, Efavirenz</td>
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<td>Lamivudin 150mg, Efavirenz</td>
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<td>Lamivudin 150mg, Efavirenz</td>
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<tr>
<td>Tenofovir and efavirenz, 9w (n=48)</td>
<td>Tenofovir 150mg, Efavirenz</td>
<td></td>
<td>Tenofovir 150mg, Efavirenz</td>
<td></td>
<td>Tenofovir 150mg, Efavirenz</td>
</tr>
</tbody>
</table>

**114** PanACEA SUDOCU COMBINATION DOSE-FINDING TRIAL SHOWS SUTEZOLID IS A SAFE OXAZOLIDINONE

**Norbert Heinrich**1, Christina Manyama2, Nyanda E. Ntinginya2, Stella Mpagama3, Alphonse Liyoyi4, Francis Mhimbira4, Benno Mbeya5, Modalakgota Sebe1, Robert Walters6, Patrick Phillips6, Leticia Wildner7, Rob Aarnoutse8, Larissa Hoffmann9, Michael Hoelscher9, Elin Svensson9

Pan African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA)

1LMU University Hospital, Munich, Germany, 2National Institute for Medical Research—Mbeya Medical Research Center, Mbeya, Tanzania, 3Kibogonio Infectious Diseases Hospital, Moshi, Tanzania, 4YaleHealth Institute, Itakyra, Tanzania, 5The Aurum Institute, Johannesburg, South Africa, 6University of California San Francisco, San Francisco, CA, USA, 7University College London, London, United Kingdom, 8Radboud University Medical Center, Nijmegen, Netherlands, 9Klinikum der Universität München, Munich, Germany

**Background:** Linezolid is a critical component of current MDR-TB treatment, but is too toxic for wider use. Sutezolid is a novel oxazolidinone, with a hypothosized improved safety profile. Sutezolid was previously only clinically tested in a 14-day monotherapy study.

**Methods:** The PanACEA Sutezolid Dose-Finding and Combination Evaluation (SUDOCU) study was a novel approach at defining the exposure-response relationship of a drug in a combination that would be usable in DS- and MDR-TB, allowing the assessment of late toxicities. Participants with drug-sensitive pulmonary TB were randomized to sutezolid 0mg, 600mg OD, 1200mg OD, 600mg BD or 800mg BD, in addition to bedaquiline, delamanid and moxifloxacin at standard doses, for 12 weeks. The primary efficacy endpoint was the slope of decline of bacterial load, measured by MGIT TTP, weekly for 12 weeks.

**Safety outcome included oxazolidinone class toxicities myelosuppression and neuropathy.**

**Results:** 75 patients were enrolled in four sites in Tanzania and South Africa. 59 (75%) were male, 2 (3%) were people living with HIV and the median weight was 53.0kg. TTP increased over 12 weeks in all arms; there was no evidence for a difference in slope between arms. No clinical neuropathy occurred during 12 weeks of treatment. One potential event of myelosuppression was noted: an HIV-coinfected patient on ARVs developed myelosuppression: neutrophil and platelet count drop of 75% of baseline. Two patients developed grade 3 seronegative hepatitis: one with jaundice and liver enzymes. 4 patients experienced a prolongation of their QTcF interval of more than 60ms over baseline, but none of them had an absolute QTcF >500ms.

**Conclusion:** Long-term NUCs effectively reduce infected hepatocytes in HBeAg-negative HIV-HBV coinfected individuals. However, infection still persists, with >10% infected cells being transcriptionally inactive. Though this data supports the ability of NUCs to reduce infection, this results underscore the limitations in eradicating HBV with NUCs alone. Future strategies to irreversibly silence cccDNA transcription may result in a functional cure.

**Change in intracellular HBV markers and transcriptional activity between biopsies**

<table>
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<tr>
<th>Participante ID</th>
<th>HBeAg</th>
<th>HBV</th>
<th>HBeAg</th>
<th>HBV</th>
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<tr>
<td>Change in intracellular markers†</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HBeAg negative</td>
<td>10.7</td>
<td>9.5</td>
<td>10.7</td>
<td>9.5</td>
<td>10.7</td>
<td>9.5</td>
<td>10.7</td>
<td>9.5</td>
</tr>
<tr>
<td>HBeAg positive</td>
<td>0.8</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**†Change in intracellular markers was defined as the difference between biopsies. Changes in intracellular HBV DNA, cccDNA, or pgRNA were defined as changes in cell.**

**Predictors of Hepatitis B treatment response in people with HIV/HBV coinfection**

Anchalee Avihingsanon1, Chee L. Leong1, Chien-Ching Hung2, Ellen Koenig3, Man-Po Lee4, Khuanchai Suppatrapinyo5, Fujei Zhang6, Hongyuan Wang7, Hal Martin8, Jason Hindman9, Jared M. Baeten10, Saisopin Kiertiburanakul10

1Thai Red Cross AIDS Research Center, Bangkok, Thailand, 2Hospital Kuala Lumpur, Kuala Lumpur, Malaysia, 3National Taiwan University Hospital, Taipei City, Taiwan (Republic of China), 4Istituto Dominicanos de Estudios Venerológicos, Santo Domingo, Dominican Republic, 5Queen Elizabeth Hospital, Kowloon, Hong Kong, 6Chung Mai University, Chang Mai, Thailand, 7Beijing Ditan Hospital, Beijing, China (People’s Republic), 8Global Science, Inc, Foster City, CA, USA, 9University of Washington, Seattle, WA, USA, 10Mahidol University, Bangkok, Thailand

**Background:** Response to hepatitis B treatment in people with HIV-1/HBV varies by baseline (BL) HBV DNA level and HBVAg status. Here we present a subanalysis of 48W outcomes from a phase 3 study (ALLIANCE) comparing bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) vs dolutegravir + emtricitabine/tenofovir disoproxil fumarate (DTG+TDF) in participants initiating treatment for HIV-1 and HBV to examine HBV DNA suppression and predictors of HBsAg loss in this coinfected population.

**Methods:** Adults with HIV-1/HBV were randomized 1:1 to initiate blinded treatment with B/F/TAF or DTG+TDF (with corresponding placebo). Co-primary endpoints were proportion with HIV-1 RNA < 50 copies/mL, SnapShot) and HBV DNA < 29 IU/mL (missing=failure) at W48. B/F/TAF was noninferior to DTG+TDF at achieving HIV-1 RNA < 50 copies/mL and superior to achieving HBV DNA < 29 IU/mL (Avihingsanon et al., AIDS 2022). Subgroup analyses determined the proportion with HBV DNA < 29 IU/mL stratified by BL HBV DNA < or = 3 log10 IU/mL and HBVAg status (+ or - at BL). A multivariate analysis (MVA) was conducted to evaluate predictors of HBV DNA < 29 IU/mL and HBsAg loss.

**Results:** 243 participants were randomized/treated (121 B/F/TAF, 122 DTG+TDF) from 46 sites globally. HBV DNA < 29 IU/mL was achieved by 53% (63%
B/F/TAF, 43% DTG+F/TDF), HBsAg loss by 9% (13% B/F/TAF, 6% DTG+F/TDF) and HBeAg loss by 20% (26% B/F/TAF, 14% DTG+F/TDF). Among those with BL HBV DNA < 8 log_{10} IU/mL (116/241), B/F/TAF-treated participants achieved significantly higher rates of HBV DNA < 29 IU/mL compared to DTG+F/TDF (67% vs 68%, \( p=0.008 \)); for those with HBV DNA ≥8 log_{10} IU/mL at BL (125/241), B/F/TAF was also numerically higher (39% vs 23%, \( p=0.037 \)). Among those who were HBeAg- at BL (187/241), B/F/TAF-treated participants achieved significantly higher rates of HBV DNA < 29 IU/mL compared to DTG+F/TDF (51% vs 31%, \( p=0.006 \)); in those who were HBeAg- (54/241), response was also numerically higher (100% vs 92%, \( p=0.055 \)). Baseline predictors of HBV DNA < 29 IU/mL from a MVA were: HBeAg-, HBV DNA < 8 log_{10} IU/mL, ALT > ULN and treatment with B/F/TAF; BL predictors of HBsAg loss included ALT > ULN and BL CD4 ≥200 cells/µL (Table 1).

**Conclusion:** In adults with HIV-1/HBV coinfection initiating therapy, B/F/TAF resulted in superior HBV DNA suppression compared to DTG+F/TDF. In MVA, B/F/TAF treatment was an independent predictor of HBV DNA suppression.

**Multivariate Logistic Regression Analysis**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient</th>
<th>CI 95%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg-</td>
<td>1.03</td>
<td>1.00 – 1.07</td>
<td>0.037</td>
</tr>
<tr>
<td>HBV DNA ≥8 log_{10} IU/mL</td>
<td>2.02</td>
<td>2.00 – 2.04</td>
<td>&lt;0.001</td>
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<tr>
<td>ALT &gt; ULN</td>
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<td>1.74 – 2.40</td>
<td>&lt;0.001</td>
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<tr>
<td>Treatment with B/F/TAF</td>
<td>2.04</td>
<td>1.74 – 2.40</td>
<td>&lt;0.001</td>
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</tbody>
</table>

**Background:** Hepatitis B virus (HBV) coinfection is common among people with HIV (PWH) and the most important cause of hepatocellular carcinoma (HCC) worldwide. Whereas the PAGE-B risk score, based on age, sex and platelets, is recommended for the prediction of HCC among individuals with HBV mono-infection, it has not been evaluated in PWH. We performed an external validation of PAGE-B in people with HIV/HBV coinfection in Europe.

**Methods:** Using data from four European cohorts (Swiss HIV Cohort Study, EuroSIDA, ATHENA and Aquitaine) we examined data in people with a positive HBsAg and without HCC before starting tenofovir. We estimated the predictive performance of the PAGE-B score on the occurrence of HCC within 15 years after tenofovir start. Model discrimination was assessed after multiple imputation using Cox regression with the prognostic index as covariate, and by calculating Harrell’s c-index. Model calibration was assessed by comparing cumulative incidence estimates using the Kaplan-Meier method with the original PAGE-B derivation study.

**Results:** In total, 2,963 individuals with HIV/HBV coinfection were included. The median age was 41 years (IQR 35–47), 466 (16%) were women, 2,023 (68%) were Caucasian, and 314 (11%) had evidence of liver cirrhosis. PAGE-B was < 10 in 26.5%, between 10 and 17 in 57.7%, and ≥ 18 in 15.7% of patients. Within a median follow-up of 9.6 years, 68 individuals developed HCC (2.58/1000 person-years, 95% CI 2.03–3.27). The regression slope of the prognostic index for developing HCC within 15 years was 0.93 (95% CI 0.61–1.25), and the pooled c-index was 0.77 (range 0.73–0.80), both indicating good model discrimination comparable to the original model derivation study. The cumulative incidence of HCC over five years was 5.6% in individuals with a PAGE-B score ≥18 in our study (Fig. A) compared to 17% in the derivation study, indicating differences in model calibration. A PAGE-B cut-off of < 10 had a negative predictive value of 99.4% for HCC within 5 years, and the HCC risk of a score < 12 remained below the commonly accepted screening threshold (Fig. B).

**Conclusion:** For individuals with HIV/HBV coinfection, PAGE-B provides a valid and accurate tool to estimate the risk of developing HCC. Individuals with a PAGE-B score < 10 are at very low risk for developing HCC. Accurate risk prediction has the potential to increase surveillance uptake in high-risk individuals, as well as reducing healthcare cost by avoiding screening of individuals with a very low HCC risk.

**Cumulative incidence of hepatocellular carcinoma**

![Graph showing cumulative incidence of HCC](image)

**Table 1: Multivariate Logistic Regression Analysis**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient</th>
<th>CI 95%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg-</td>
<td>1.03</td>
<td>1.00 – 1.07</td>
<td>0.037</td>
</tr>
<tr>
<td>HBV DNA ≥8 log_{10} IU/mL</td>
<td>2.02</td>
<td>2.00 – 2.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT &gt; ULN</td>
<td>2.04</td>
<td>1.74 – 2.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment with B/F/TAF</td>
<td>2.04</td>
<td>1.74 – 2.40</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Background:** Oral doxycycline (DOX) prophylaxis is a promising strategy to prevent bacterial sexually transmitted infections (STIs). Clinical trials demonstrated a 200 mg oral DOX dose taken by men who have sex with men (MSM) after sexual exposure can provide protection against STIs. However, mucosal pharmacokinetic data at the site of STI exposure and DOX activity are lacking. We examined mucosal DOX concentrations in men and women to better understand DOX efficacy and inform dose optimization for STI prevention.

**Methods:** Eleven male and 9 female participants provided blood and mucosal swabs up to 7 days after receiving a 200 mg oral DOX dose. Rectal, vaginal and cervical biopsies as well as urethral swabs were collected 24 hours after dosing. DOX was measured by liquid chromatography–mass spectrometry with a lower limit of quantification of 10 ng/mL for plasma and 2.5 ng/sample for swabs and biopsies. Secretion concentrations were estimated using swab weight. Concentrations are reported as geometric mean and 95% confidence interval. Pharmacologic data on mucosal DOX exposures associated with STI protection are lacking. This study provides the first pharmacologic data on mucosal DOX exposures associated with STI protection among MSM, predicts high vaginal efficacy, and informs a rational DOX dose optimization for STI prevention in men and women.

**Results:** Rectal secretion DOX concentrations peaked at 48 hours, 8 hours in vaginal secretions, and 4 hours in plasma. Rectal and vaginal DOX exposure up to 96 hours were 2- and 3-times that of plasma, respectively. Rectal and vaginal secretion DOX concentrations remained above the MIC_{90} for 48, 72 and 96 hours, longer than in plasma, for NG, TP and CT, respectively. DOX concentrations in rectal (616 ng/g; 495 – 766 ng/g), vaginal (261 ng/g; 98 – 696 ng/g) and cervical (410 ng/g; 193 – 870 ng/g) were only 1- to 2-times the MIC_{90} for NG, but at least 2- to 4-times greater than the MIC_{90} for TP and CT, respectively. Urethral secretion DOX was estimated to be at least 4-times the MIC_{90} for NG, TP and CT, and greater than plasma or mucosal concentrations.

**Conclusion:** DOX efficiently distributes to mucosal sites and maintains inhibitory concentrations against TP and CT for 3-4 days after dosing, but only 2 days for NG which may impact level of protection. This study provides the first pharmacologic data on mucosal DOX exposures associated with STI protection among MSM, predicts high vaginal efficacy, and informs a rational DOX dose optimization for STI prevention in men and women.
ANRS 174 DOXYVAC: AN OPEN-LABEL RANDOMIZED TRIAL TO PREVENT STIs IN MSM ON PrEP
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Cécile Bebear3, Nicolas Dupin1, Jean-Paul Viard4, Juliette Pavie1, Claudine  
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Bordeaux, France, 4Hôtel-Dieu de Paris, Paris, France

Background: Increased rates of sexually transmitted infections (STI) are reported among men who have sex with men (MSM), in particular those using pre-exposure prophylaxis for HIV (PrEP). Interventions to reduce STI incidence are needed.

Methods: MSM on PrEP with a history of STI in the past year, were randomized in an open-label factorial design trial to receive doxycycline post-exposure prophylaxis (DoxY-PEP: 200 mg within 72h of condomless sex) or no PrEP (2:1); and 2 shots of meningococcal B vaccine (Bexsero®) 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. Serologic tests for syphilis were performed every 3 months. A committee adjudicated STI blinded to study arms. The co-primary endpoints were: the incidence of first episode of CT or syphils for Doxy PEP and the incidence of a first episode of GC, 1 month after the second injection, 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. Results for data collected up to July 15, 2022 are presented.

Results: Between January 19, 2021, and July 15, 2022, 546 MSM were randomized and 502 were analyzed. Median age: 39 years, median of 10 sexual partners in past 3 months. Median follow-up: 9 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 5.6 and 35.4 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.16; 95%CI: 0.08-0.30). The incidence of a first episode of GC was 20.5 and 41.3 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.49; 95%CI: 0.32-0.76). The incidence of a first episode of GC was 9.8 and 19.7 per 100 PY in the meningococcal B vaccine and no vaccine arms, respectively (aHR: 0.49; 95%CI: 0.27-0.88). No drug-related SAE was reported.

Conclusion: Among MSM on HIV PrEP, doxycycline PEP significantly reduced the incidence of CT and syphilis and also had a significant impact on the incidence of GC. Meningococcal B vaccine also reduced the incidence of GC.

DOXYPEP & ANTIMICROBIAL RESISTANCE IN N. GONORRHOEAE, COMMENSAL NEISSERIA & S. AUREUS
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Background: Doxycycline post-exposure prophylaxis (doxy-PEP) is highly effective in reducing N. gonorrhoeae (GC), C. trachomatis (CT), and syphilis among men who have sex with men (MSM) and transgender women (TGW). Understanding the effect of doxy-PEP use on antimicrobial resistance (AMR) in N. gonorrhoeae and bacteria which can cause disease (S. aureus) or transmit resistance (Neisseria spp) is unknown.

Methods: DoxyPEP is a randomized open-label trial among MSM/TGW living with HIV on PrEP with GC, CT, or early syphilis in the past year; participants were randomized 2:1 to 200 mg doxycycline within 72 hours of condomless sex or no doxycycline (SOC). At months 0 (M0) and 12 (M12), naso/oropharyngeal swabs were cultured for S. aureus (SA) with doxycycline resistance (doxy-R) defined as MIC ≥16 µg/ml by E-test and oropharyngeal swabs cultured for commensal Neisseria (doxy-R: MIC ≥2 µg/ml by E-test). Participants with a positive GC test were instructed to return for swabs for GC culture through the CDC SURRG program (TCN-R: MIC≥2.0 µg/ml by agar dilution). Overall proportion with growth or AMR at M12 were compared by Fisher’s exact test.

Results: Of 501 participants as of May 2022, 5 were cultured at M0 in 44.2% and doxy-R SA in 6.3% (Table). At M12, 5 were cultured from 29.2% in the doxy-PEP arm and 45.2% in the SOC arm (p=0.036), with doxy-R SA present in 11.7% and 4.8% (p=0.19), respectively. At M0, methicillin-resistant-SA (MRSA) was cultured from 5.9%, and at M12, 1.5% in the doxy-PEP arm and 6.5% in the SOC arm (p=0.077). At M0, Neisseria spp were cultured from 66.8% with doxy-R Neisseria in 61.8%. At M12, Neisseria spp were cultured from 82.2% in the doxy-PEP arm and 89.3% in the SOC arm (p=0.64), and doxy-R was 69.7% and 44.6%, respectively (p=0.017). Among GC diagnoses, 17% (44/256) had phenotypic susceptibility results; M0 TCN-R was 28.4% (4/145), and after enrollment, 38.5% (5/13) in the doxy-PEP arm and 12.5% (2/16) in the SOC arm.

Conclusion: Doxy-PEP reduced S. aureus colonization by 16% without a significant increase in doxy-R SA. A majority had doxy-R commensal Neisseria at baseline, with an unexpected decrease in doxy-R Neisseria spp in the SOC arm. These modest changes in doxy-R S. aureus and Neisseria spp are unlikely to have clinical significance and must be considered in context of >60% STI reduction with doxy-PEP. Doxy-PEP may be less protective against incident TCN-R GC; surveillance for the impact of TCN-R GC on doxy-PEP efficacy and doxy-PEP on GC resistance is needed.

S. aureus, Neisseria spp, and N. gonorrhoeae: Bacterial isolation and phenotypic resistance to the tetracycline antibiotic class at enrollment and during DoxyPEP study

Doxycycline postexposure prophylaxis for prevention of STIs among cisgender women
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Background: Bacterial sexually transmitted infections (STIs) are responsible for significant and disproportionate morbidity and mortality in cisgender women, among whom STI incidence has been rising globally. Doxycycline taken as postexposure prophylaxis (PEP) was efficacious at preventing STIs among cisgender men who have sex with men and transgender women, but no trials among cisgender women have been done.

Methods: We conducted an open-label randomized trial of doxycycline PEP (doxycycline hydrate 200mg taken within 72 hours of sex) compared with standard of care (e.g., quarterly screening and treating STIs) among women aged 18-30 years in Kisumu, Kenya. Participants were required to already be taking daily oral HIV preexposure prophylaxis (PrEP). Contraception was not required, and doxycycline was stopped during pregnancy. Weekly SMS surveys 2

Results: We enrolled 449 cisgender women; women completed 97% of expected follow-up visits. Median age was 24 years (IQR 21-27); 36.7% reported transactional sex at enrollment, and baseline STI prevalence was 17.9% (14.1% C. trachomatis, 3.8% N. gonorrhoeae, 0.4% T. pallidum). Incident STI events were detected at 109 follow-up visits (85 C. trachomatis, 31 N. gonorrhoeae, 121
122 POTENTIAL IMPACT AND EFFICIENCY OF DOXY-PEP AMONG PEOPLE WITH OR AT RISK OF HIV
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Background: Doxycycline post-exposure prophylaxis (doxy-PEP) reduces bacterial sexually transmitted infection (STI) risk in people with HIV (PWH) or using HIV preexposure prophylaxis (PrEP). However, ongoing decision-making about federal and local guidance for doxy-PEP prescribing is complicated by concerns about potential harms of widespread use. We sought to identify doxy-PEP prescribing strategies that would minimize overall doxy-PEP use while maximizing impact on STIs.

Methods: We used electronic health record data on gay and bisexual men (GBM), transgender women, and non-binary people assigned male at birth with ≥2 STI tests (chlamydia, gonorrhea, or syphilis) at Fenway Health, a Boston clinic focused on LGBT health, during 2015-2020. Patients were followed from first STI test until last test or end of 2020. We defined 10 potential doxy-PEP prescribing strategies: prescribed to (1) all individuals, (2) PrEP users, (3) PrEP users/PWH; and prescribed after (4) any STI, (5) rectal STI, (6) ≥2 STIs in 12m, (7) ≥2 STIs in 6m, (8) ≥2 concurrent STIs, (9) syphilis or (10) gonorrhea Dx. We also explored strategies 4–10 restricted to PrEP users/PWH. We evaluated counterfactual scenarios in which patients who met each criterion were prescribed doxy-PEP indefinitely (strategies 1–3) or for 12m (strategies 4–10). We assumed STI incidence during doxy-PEP use would have been reduced by clinical trial efficacy estimates. For each strategy, we estimated the proportion prescribed doxy-PEP, overall STIs averted, and number needed to treat with doxy-PEP per year to avert 1 STI (NNT).

Results: Among 10,562 patients (94% GBM; 54% PrEP users; 14% PWH), incidence of any STI was 39.8/100py. Across strategies, NNT ranged from 1.0–3.8 (median=1.7) and proportion of STIs averted ranged from 8%–70% (median=20%). Prescribing doxy-PEP to all patients averted 70% of STIs (NNT=3.7); prescribing to PrEP users/PWH (68% of patients) averted 60% of STIs (NNT=2.9; Figure). Prescribing doxy-PEP after any STI reduced the proportion of patients on doxy-PEP to 41% and averted 42% of STIs (NNT=2.9). Prescribing after concurrent or repeated STIs was most efficient (lowest NNTs). Restricting strategies 4–10 to PrEP users/PWH had minimal effect on NNT and reduced impact on STIs.

Conclusion: Prescribing doxy-PEP to individuals with STIs, particularly concurrent or repeated STIs, could avert a substantial proportion of subsequent STIs. The most efficient prescribing strategies are based on STI history rather than HIV status or PrEP use.

123 CLUSTER RANDOMISED TRIAL OF RISK-DIFFERENTIATED CARE FOR FEMALE SEX WORKERS: AMETHIST
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1Liverpool School of Tropical Medicine, Harare, Zimbabwe, 2Centre for Sexual Health and HIV/AIDS Research Zimbabwe, Harare, Zimbabwe, 3London School of Hygiene & Tropical Medicine, London, United Kingdom, 4University College London, London, United Kingdom, 5Africa Health Research Institute, Durban, South Africa, 6Liverpool School of Tropical Medicine, Impensa, Italy, 7National AIDS Council, Harare, Zimbabwe, 8Ministry of Health and Child Welfare, Harare, Zimbabwe

Background: Strengthening outreach and community mobilisation are promising approaches for reducing HIV transmission attributable to sex work.

Methods: We allocated 22 clusters using restricted random allocation (1:1) to usual care or AMETHIST interventions. A cluster was the female sex worker (FSW) population around a clinic providing the services listed below.

Usual care: FSW-friendly services, HIV testing, PrEP/ referral to government ART services, contraception, condoms, STI syndromic management, health education and legal advice; all supported by peer educators.

AMETHIST:usual care plus peer-led microplanning (outreach tailored to address individual vulnerability) and participatory self-help groups to support risk-differentiated HIV prevention, testing, ART/PrEP and adherence.

To outcome: proportion of all FSW at risk of HIV transmission (HIV-positive, not virally suppressed and not consistently using condom) or HIV acquisition (HIV-negative and not protected by condoms or PrEP).

Results: AMETHIST was implemented June 2019-October 2021. During this period there were more clinic registrations, HIV tests and PrEP/ ART initiations at AMETHIST compared to Usual care sites.

4444 FSW aged 18+ were enrolled in the endline survey, providing questionnaire data, biospecimens (n=4443) including dried blood spot (HIVab, HIV viral load), plasma (TDF levels) and self-taken vaginal swabs for STI and Y-chromosome testing (to validate condom use reports). 46.2% were living with HIV of whom 1790/1973 (90.7%) were virologically suppressed. 586/2334 (25.1%) of the HIV negative women reported currently taking PrEP, but only 2 had plasma TDF levels >700nmol/punch.

There was no impact on the primary endpoint of population level risk of HIV transmission/acquisition. The proportion of HIV positive FSW with a viral load >1000copies/ul was low in both arms, and significantly reduced in the AMETHIST intervention arm. There was no impact of AMETHIST on risk of HIV acquisition.

See Table 1 AMETHIST.

Conclusion: Risk of HIV transmission in HIV positive women was low (95:95:95 achieved) and further improved by the AMETHIST Intervention. Risk of HIV acquisition was high and unchanged. Sustaining testing and treatment and strengthening HIV prevention including through long-acting PrEP is critical.
124 RANDOMIZED TRIAL OF COMMUNITY HEALTH WORKER DELIVERED DYNAMIC CHOICE HIV PREVENTION

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SEARCH Study Team

Infectious Diseases Research Collaboration, Kampala, Uganda; Kenya Medical Research Institute, Kisumu, Kenya; University of California San Francisco, San Francisco, CA, USA; National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; Makerere University, Kampala, Uganda; University of California Benecery, Berkeley, CA, USA

Background: Maximizing HIV prevention coverage requires community entry points and structured models for delivering patient-centered choices over time. We hypothesized that a dynamic choice prevention (DCP) intervention, including flexibility to move between PrEP and PEP and delivered by community health workers (CHW), would increase HIV biomedical prevention coverage among persons at risk in rural Sub-Saharan Africa.

Methods: We conducted a cluster randomized trial among persons (≥15 years) with current or anticipated risk of HIV in Uganda and Kenya (SEARCH; NCT04810650). Intervention villages received DCP delivered by CHW with clinician support. DCP included: 1) product choice (daily oral PrEP [TDF/FTC] vs. PEP), 2) access to self-testing (FTPPE) with the option to switch over time, 2) service location choice, 3) HIV self-testing option, 4) 24/7 phone access to clinician, and 5) CHW guidance on patient-centered care. Control villages received standard care prevention referrals. The primary outcome was biomedical prevention coverage: proportion of 48-week follow-up with self-reported PrEP/PEP use. Coverage during self-reported HIV risk periods was a secondary outcome. Arms were compared using TMLE, accounting for clustering.

Results: From May-July 2021, we enrolled 429 persons (212 intervention; 217 control) in 16 villages; 57% were women and 35% aged 15–24 years. 58% of intervention participants chose PrEP and 58% chose PEP at least once over 48 weeks. Choice of self-testing increased from 52% at baseline to 71% at week 48. Choice of out-of-facility (vs. clinic) delivery was ≥98% throughout. Among persons at risk in rural Sub-Saharan Africa.

125 CLUSTER RANDOMIZED TRIAL OF INTEGRATED HIV SEXUAL & REPRODUCTIVE HEALTH SERVICES

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CHIEDZA Trial Group

London School of Hygiene & Tropical Medicine, London, United Kingdom; Biomedical Research and Training Institute, Harare, Zimbabwe; The University of Sydney, Sydney, Australia; Ministry of Health and Child Welfare, Harare, Zimbabwe

Background: Youth living with HIV (YLWH) have disproportionately poor virological outcomes. We hypothesized that a community-based intervention incorporating the whole HIV cascade (testing, treatment, adherence support) integrated with sexual & reproductive health (SRH) services, would improve HIV outcomes among youth. Integrating SRH may increase engagement in a hard-to-reach group with limited access to health services.

Methods: A cluster randomized trial (CHIEDZA) was conducted across 3 provinces in Zimbabwe, each randomized 4:4 to control (existing services) or to intervention clusters (total 24 clusters). A cluster was a geographically demarcated area with a community center, from where weekly integrated HIV & SRH services were delivered to intervention cluster residents aged 16–24 years. The primary outcome was virological suppression (VS) (<1000 copies/ml) among YLWH after 30 months, ascertained through a population-based survey of 700 18-24 year olds/clusters (total 16,800). Secondary outcomes assessed each step of the HIV cascade.

Results: 36,991 youths accessed the CHIEDZA intervention (estimated 95% of eligible population in intervention clusters), of whom 84% had ≥1 HIV test. The improvement in the YLWH CHIEDZA intervention (24% newly diagnosed), 1445 (94%) were linked to HIV care of whom 1409 (97%) were taking ART, of 815 on whom VL was available, 80% had VS. The population-based survey enrolled 17682 (95% of those eligible), 60.7% female; 47.7% aged 21-24 years. In the intervention arm, 29% reported accessing CHIEDZA. HIV prevalence was 5.9% and 7.5% in intervention and control arms respectively. A higher proportion in the intervention than control arm resulted having an HIV test (71% vs 66.1%, p=0.016) and knowledge of HIV status (68.5% vs 63.3%; p=0.057). There was no difference by arm in the primary outcome (40% vs 37.6%; RR 1.05 (95% CI 0.98–1.12), or in secondary outcomes. Among older YLWH taking ART, VS was higher in the intervention than the control arm (interaction p=0.017) (Table 1).

Conclusion: Despite high levels of HIV testing and treatment achieved by CHIEDZA, there was no population level impact on VS. While the intervention did increase knowledge of HIV status among youth at population level, those at highest HIV risk were not identified, which likely explains lack of intervention effect. Mobility explains the 29% reported coverage at endline (despite 95% eligible population served). Nearly 50% of YLWH remain undiagnosed; strategies to address this are urgently needed.

Table 1: Primary and secondary outcomes stratified by age and sex

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Cluster-level geometric mean</th>
<th>Risk Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36.5%</td>
<td>3.00 (2.87-3.15)</td>
<td>0.01</td>
</tr>
<tr>
<td>Female</td>
<td>32.8%</td>
<td>2.84 (2.68-3.01)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age ≥ 18</td>
<td>37.8%</td>
<td>2.88 (2.71-3.06)</td>
<td>0.01</td>
</tr>
<tr>
<td>Secondary outcomes by sex (adjusted for age in years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV status</td>
<td>68.5% (63.3%)</td>
<td>1.05 (0.98–1.12)</td>
<td>0.01</td>
</tr>
<tr>
<td>Taking ART (%)</td>
<td>56.6% (50.2%)</td>
<td>1.04 (0.95-1.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>YLWH (%)</td>
<td>54.9% (50.7%)</td>
<td>1.07 (0.99-1.14)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

126 RECENT HIV INFECTED WOMEN IN BREASTFEEDING IN MALAWI: JUL 2019-JUL 2022

Davie D. Chalira1, Bridget Miller1, Danielle Payne2, Melissa Aros2, Alinune N. Kabaghe3, Joe Theu4, Romane Thawi5, Reno Stephens4, Ireen Namakhoma4, Joseph Njala5, Alexandra Ernst6, Gabrielle O’Malley7, Kerry A. Thomson7, Nellie Wandonda8, Kashongwe9

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Background: HIV infections can be characterized as recent (acquired within the previous 12 months) using a rapid test for recent infection (RTRI) and a viral load (VL) ≥1,000 copies/ml. As part of a recent infection testing algorithm (RITA), Recent HIV infection surveillance (RIS) aims to identify geographic areas and subpopulations where potential transmission is occurring in order to guide public health response. To better understand recent infections among breastfeeding women (BF) across Malawi, we designed a survey to assess breastfeeding HIV testing services (BHTS), we evaluated data from Malawi’s RIS system.

Methods: We analyzed RIS data from July 2019 to July 2022, among individuals ≥13 years with an HIV-positive result, from 27 districts in Malawi. Proportions of RITA-recent infections were calculated by dividing RITA-recent cases by the estimated RIS denominator.

Table 2: RITA-recent infections in breastfeeding women in Malawi

<table>
<thead>
<tr>
<th>Variable</th>
<th>RITA-recent infections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malawi (%)</td>
<td>36.5 (34.1-38.8)</td>
</tr>
<tr>
<td>Gender Male (%)</td>
<td>31.7 (29.3-34.2)</td>
</tr>
<tr>
<td>Gender Female (%)</td>
<td>40.9 (38.4-43.4)</td>
</tr>
<tr>
<td>Age 13-17 years (%)</td>
<td>32.5 (29.9-35.1)</td>
</tr>
<tr>
<td>Age 18-24 years (%)</td>
<td>40.9 (38.4-43.4)</td>
</tr>
</tbody>
</table>

11 AMETHIST
valid RTRI results. Proportions were calculated by demographic characteristics and statistically significant differences were identified using chi-square tests (p<0.05).

Results: Among 55,347 newly diagnosed HIV-positive clients with a RTRI result, 666 (1.2%) were BFW. The median age of BFW was 25 (IQR: 22-30). Fifty-three BFW (8.8%) had a recent HIV infection compared to 2.0%, 3.6%, and 3.4% (p<0.00) of males, non-pregnant females, and pregnant females, respectively. By age group, the proportion RTI-positive among BFW was 8.9%, 7.1%, 8.5%, and 0.8% among 15-24yo, 25-34yo, 35-44yo, and 45-54yo, respectively. Regarding HTS entry points, the maternal and child health/under 5, maternity, and post-natal entry points accounted for 32.1% of RTI-positive BFW, whereas voluntary testing and counseling accounted for 45.3%. Among RTI-positive BFW, 47 (88.7%) self-reported their last HIV test result as negative and 3 (5.7%) as inconclusive. Among RTI-positive BFW with a recent negative result, 93.6% received their last HIV test within the past year and 61.7% within the past 6 months.

Conclusion: RTI in Malawi identified about one recent infection out of every eight newly diagnosed HIV+ breastfeeding women, although BFW contribute < 2% of new diagnoses at HTS. The majority of RTI-positive BFW tested negative within the past 6 months. This suggests that while antenatal testing programs have high coverage, risk of HIV acquisition persists throughout pregnancy and breastfeeding, therefore prevention strategies could be enhanced for this group. Future analyses that investigate risk factors and geographic variation could inform counseling messages, partner engagement, and prevention options.

127 DELIVER: A SAFETY STUDY OF A DAPIVIRINE VAGINAL RING AND ORAL PrEP DURING PREGNANCY

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MTN-042 DELIVER Study Team

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Background: Pregnancy represents a high-risk period for HIV acquisition. The monthly dapivirine vaginal ring (DVR) has been clinically shown to reduce HIV risk with no safety concerns in nonpregnant reproductive-aged cisgender women; however, data during pregnancy are limited. Here we report safety data from the first two cohorts of pregnant participants in MTN-042/DELIVER, a phase 3b, randomized, open-label safety trial of DVR and oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) (MTN-042/DELIVER, NCT03965923.)

Methods: Eligible pregnant individuals aged 18 to 40 in Malawi, South Africa, Uganda, and Zimbabwe were randomized 2:1 to monthly DVR or daily TDF/FTC. Participants in cohort 1 initiated product use between 36 0/7-37 6/7 weeks gestation; in cohort 2 product use was started at 30 0/7-35 6/7 weeks gestation. All participants continued product use until delivery or 41 6/7 weeks gestation. Pregnancy outcomes and complications reported at the time of delivery were assessed and summarized using descriptive statistics and compared to local background rates obtained through a systematic chart review (MTN-042B).

Results: One-hundred and fifty participants were enrolled into cohort 1 with 101 randomized to DVR and 49 to TDF/FTC. One-hundred and fifty-seven participants were enrolled into cohort 2 with 106 randomized to DVR and 51 to TDF/FTC. Demographic and clinical characteristics were similar by study arm for each cohort. In cohort 1, one stillbirth and one neonatal death occurred, both in the DVR/FTC arm. One stillbirth and one neonatal death occurred in cohort 2, both in the DVR arm. The prevalence of preterm delivery was 2% in cohort 1 and 6% in cohort 2. In both cohorts, pregnancy complications were rare, with hypertensive disorders being the most commonly reported, and generally similar to local background rates (Table 1). There were no cases of fever of unclear etiology or preterm premature rupture of membranes reported. In cohort 2, there was 1 (1%) case of choioamnionitis in the DVR arm and 1 (1%) of endometritis in the TDF/FTC arm, and 2 (4%) of puerperal sepsis in the TDF/FTC arm.

Conclusion: In this first study of a long-acting HIV prevention agent in pregnancy, adverse pregnancy outcomes and complications were uncommon when DVR and TDF/FTC were used in the third trimester of pregnancy and were similar to rates observed in the communities where the study is being conducted. These data support plans for subsequent investigation of DVR safety earlier in pregnancy.

Maternal pregnancy complications by study arm

<table>
<thead>
<tr>
<th>Pregnancy Complication</th>
<th>MTN-042 Cohort 1</th>
<th>MTN-042 Cohort 2</th>
<th>Local background rates in MTN-042 (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihypertensive disorder or hypertension</td>
<td>2 (2%)</td>
<td>9 (9%)</td>
<td>3 (3%) - 10% - 22.5% (9/40, 12)</td>
</tr>
<tr>
<td>Cardiac/vascular failure</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>3 (3%) - 9 (9%) - 6.0% (9/14)</td>
</tr>
<tr>
<td>Pre-eclampsia without severe features</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>2 (2%) - 4 (4%) - 4.2% (4/92)</td>
</tr>
<tr>
<td>Pre-eclampsia with severe features</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>6 (6%) - 6 (6%) - 6.0% (6/100)</td>
</tr>
<tr>
<td>N/A</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%) - 13 (13%) - 1.6% (13/798)</td>
</tr>
<tr>
<td>Peripartum/Artificial birth</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
<td>2 (2%) - N/A - N/A</td>
</tr>
</tbody>
</table>

128 RANDOMIZED TRIAL OF DYNAMIC CHOICE HIV PREVENTION IN ANTE-/ POSTNATAL CARE CLINICS

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Background: Women seen for ante and postnatal care (ANC) remain at risk for HIV in rural sub-Saharan Africa, despite routine access to PrEP. Patient-centered prevention delivery models that offer structured choices in product, testing and visit location may increase coverage. Few studies evaluate actual prevention choices made by clients, nor the impacts of a dynamic choice delivery model on biomedical prevention coverage.

Methods: We conducted an individually randomized trial (SEARCH; NCT04810650) among women (≥15 years) with current or anticipated risk of HIV infection seen at ANC clinics in rural Kenya and Uganda to evaluate the effect of a dynamic choice prevention (DCP) model (intervention) versus standard-of-care (control). DCP included: 1) product choice (daily oral PrEP [TDF/FTC] or post-exposure prophylaxis [PEP]) with option to switch over time; 2) service delivery model choice; 3) HIV self-testing option; 4) 24/7 phone access to clinician; and, 5) provider training on patient-centered care. The primary outcome was biomedical prevention coverage over 48 weeks (proportion of months with self-reported PrEP or PEP use); self-reported use during months a client retrospectively reported HIV risk was a secondary outcome.

Results: We enrolled 400 women between April and July 2021 (203 intervention, 197 control); 38% were pregnant; 94% reported no PrEP or PEP use for prior 6 months; 52% were aged 15-24y. Among 384/400 (96%) of women with outcome ascertained, the intervention increased biomedical prevention coverage by 40.2% (95% CI: 33.8%-46.7%; p< 0.001); mean coverage was 70% in intervention vs. 29% in control. Similar effect sizes were seen across age and baseline pregnancy status. The intervention also increased coverage during months at risk of HIV: 81% in intervention vs. 43% in control (38.1% absolute increase; 95% CI: 31.2%-45.0%; p< 0.001). Among intervention participants, 100% chose PrEP and 11% chose PEP at least once during 48 weeks. Choice of off-site visits increased over time (61% selected out-of-facility delivery at week-48 vs. 22% at baseline), as did choice of HIV self-testing (59% selected self-testing at week-48 vs. 34% at baseline).

Conclusion: In this randomized study, a patient-centered dynamic choice intervention that provided flexibility in product modality, testing and service location more than doubled biomedical HIV prevention coverage in a high-risk population already routinely offered access to biomedical prevention options.
129 MATERNAL POINT-OF-CARE VIRAL LOAD AT DELIVERY IMPACTS INFANT ARV PROPHYLAXIS REGIMEN


The LIFE Study Consortium

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Background: Despite tremendous achievement in reducing HIV mother-to-child transmission (MCTC), 150,000 children were newly infected in 2020 globally. High maternal viral load (VL) at delivery is among the strongest risk factors for MCTC. Currently, the choice of postnatal antiretroviral prophylaxis (PNP) for HIV-exposed infants is based on WHO high-risk (HR) criteria. We aimed to estimate the contribution of point-of-care (PoC) maternal VL testing at delivery in profiling the risk of MCTC and its impact on standard PNP or enhanced PNP (ePNP) for HIV-exposed infants.

Methods: The cluster-randomized LIFE trial was conducted at 28 health facilities in Tanzania and Mozambique. At delivery, the intervention arm A provided PoC maternal VL to aid MCTC HR assessment in addition to clinical criteria and antenatal care information as available in the control arm B only. In Tanzania both arms initiated ePNP based on maternal risk factors, including VL for arm A, while in Mozambique, ePNP was provided universally. We used mixed effects logistic regression models to estimate the effect of our intervention on the proportion of infants 1) identified as HR (Tanzania and Mozambique) and 2) HR infants receiving ePNP (Tanzania only). Standard errors were clustered to account for health facility and multiple births.

Results: A total of 6512 mothers living with HIV were enrolled: 72% were diagnosed before the 2nd trimester, 95% were on ART, and 78% were virally suppressed at delivery. Of 6668 newborns, 781 (12%) were classified as HR (636 (9.5%) vs. 145 (4.4%) in arms A and B, respectively; p < 0.0001). In arm A, 512 (80.5%) HR infants were classified only based on maternal PoC VL at delivery. In arm B, overall 609 (18.4%) additional infants would have been identified as HR if their mothers would have received PoC VL assessment. In Tanzania, HR infants in arm A were significantly more likely to receive ePNP, with 67/112 (59.8%) vs. 16/51 (31.4%) receiving ePNP in arms A and B, respectively (OR 4.49, 95% CI: 1.23, 16.36). However, 40.2% in arm A and 68.6% in arm B did not receive ePNP despite available information for HR classification at delivery.

Conclusion: PoC maternal VL testing at delivery significantly increased the proportion of infants identified as HR. Further, HR infants with maternal PoC VL at delivery were more often initiated on ePNP. However, linkage of HR infants to appropriate antiretroviral prophylaxis remains suboptimal, warranting consideration of universal ePNP irrespective of transmission risk.

130 MULTICOMPONENT INTERVENTION IMPROVES VIRAL SUPPRESSION FOR PREGNANT/PART-TUM WOMEN

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Background: Achieving viral suppression among pregnant and breastfeeding women is essential to eliminate vertical transmission of HIV in Sub-Saharan Africa. We hypothesized that an enhanced viral load counseling and standardized peer-mother support (ENHANCED-SPS) intervention would increase viral suppression among pregnant and breastfeeding women in rural Uganda.

Methods: This was a cluster randomized trial evaluating the effect of the ENHANCED-SPS intervention among pregnant and post-partum women receiving HIV care at 14 facilities in rural SouthWestern Uganda (NCT0412214-4). Intervention participants received point-of-care viral load testing and enhanced viral load counseling during clinic visits along with bi-weekly phone counseling from a trained peer-mother over a period of 12 months. Within the intervention arm, we compared the proportion with viral suppression (HIV RNA < 1000 c/mL) at baseline and 12 months, accounting for clustering and missing measures. Endpoint data were not available in the control arm, preventing a by-arm comparison.

Results: We enrolled 505 pregnant and post-partum women at the 7 intervention clinics from September 2019 to October 2020. Participants’ median age was 28 years, 157/505 (31%) were newly diagnosed with HIV, 77% (388/505) were on an EFV-based regimen and 70% (553/78%) were virally suppressed. After 12 months of the ENHANCED-SPS intervention, viral suppression was 95% (95% CI: 93%-97%), corresponding to a 25% (95% CI: 22-28%; p < 0.001) absolute increase from the baseline measurement. The intervention increased suppression within subgroups of age, ART regimens, and enrollment group, with the greatest increase among those newly diagnosed: 70% increase (95% CI: 63-77%) among pregnant women and 59% increase (95% CI: 27-90%) among breastfeeding women.

Conclusion: The multi-component, peer-led intervention significantly increased virologic suppression within 1 year of implementation. Despite the observed increase following the intervention, viral suppression among breastfeeding women remains far below the target rates, highlighting the need for additional interventions during the postpartum period.

Viral suppression (< 1000 c/mL) among the intervention participants

131 IMPROVED STRATEGY TO PREVENT HIV POSTNATAL TRANSMISSION: A RANDOMIZED TRIAL

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Background: HIV transmission through breastfeeding remains a bottleneck to eliminate pediatric HIV infections in Africa. We aimed to assess the efficacy of a combined intervention including maternal viral load (VL) point of care (POC) testing and extended infant postnatal prophylaxis (PNP) to prevent postnatal transmission at 12 months in Lusaka (Zambia), Ouagadougou and Bobo-Dioulasso (Burkina Faso).

Methods: Breastfed HIV-exposed infants were enrolled at the 2nd infant immunization visit (EPI-2, 6-8 weeks). A POC HIV DNA was done at EPI-2, and HIV-infected infants were promptly initiated on ART. HIV-uninfected infants (HEU) infants and their mothers were then randomized to two arms. In the intervention arm, mothers had a POC VL testing during EPI-2 and M6 visits. Infants whose mothers had a VL >1000 c/mL were initiated on lamivudine (3TC) until M12 or until 8 weeks after cessation of breastfeeding. The control group implemented PMTCT activities as per local WHO-derived guidelines. The primary outcome was infant HIV transmission at M12. Secondary outcomes were safety and the period at high risk of transmission defined as no PNP and a maternal VL >1000 c/mL while breastfeeding.

Results: From Dec 2019 to Sep 2021, 1526 HIV-exposed infants were enrolled and had HIV diagnosis at EPI-2. Among them, 20 infants were newly diagnosed with HIV, started prompt ART, and 1506 HEU infants (1342 from Zambia and 164 from Burkina Faso) were randomized (753 in each arm). Baseline characteristics were similar between arms. The mothers had a median age of 30.6 years (IQR:26.0-35.0), and 98.4% were on ART. In the intervention arm, the POC VL prompted 3TC initiation for 102 infants (85 at EPI-2 and 17 at M6). The period at high risk for transmission was 0.47/100 person-days (95% CI: 0.44-0.50) in the intervention arm vs. 6.58/100 person-days (95% CI: 6.47-6.70) in the control arm.

Improving postnatal transmission prevention: a randomized trial.
control arm. Within 12 months of follow-up, 1 HIV transmission occurred in the intervention arm vs. 6 in the control arm, yielding transmission rates of 0.19/100 person-years (95% CI: 0.05-0.50) and 1.17/100/person-years (95% CI: 0.43-2.55), respectively. The rate of SAE was similar in both arms.

Conclusion: Our randomized controlled trial showed that an intervention nested in EPI and combining POC measurement of maternal viral load and, when unsuppressed, same-day infant single PNP initiation was safe and reduced the period at high risk of more than 90%, which resulted in a sharp reduction (though not significant) of HIV transmission at 12 months.

132 BIRTH POINT-OF-CARE TEST & TREAT REDUCES EARLY MORTALITY AMONG HIV INFECTED INFANTS


LIFE Study Consortium

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Background: Early mortality among infants with HIV is high and peaks at 2-3 months of age. Late diagnosis delays access to antiretroviral treatment (ART), often past the mortality peak. We assessed the impact of a point-of-care (PoC) HIV early infant diagnosis (EID) test & treat strategy at birth on viral suppression and mortality up to 24 weeks of age among HIV-infected infants in Mozambique and Tanzania.

Methods: We conducted a cluster-randomized trial at 28 public health facilities. Intervention arm A sites (n=14) provided PoC EID and immediate nurse-directed, physician-supported ART initiation for positive infants at birth, while control arm B sites (n=14) offered PoC EID and linkage to ART from 4-6 weeks of age. Infant ART at birth included nevirapine-based regimens, and by 4-6 weeks infants were switched or started on lopinavir/ritonavir granule-based regimens. Study visits were conducted at birth, 4-6 weeks, 12 weeks and 24 weeks. The Kaplan-Meier method was used to compare survival between arms. Mixed-effects Cox proportional-hazards models adjusted for time of HIV infection with standard errors clustered at the health facility level were used to estimate hazard ratios (aHR). Proportions of infants virally suppressed (< 1000 copies/ml) at 24 weeks of age between arms are reported.

Results: Among 6606 infants enrolled, 3298 in arm A and 3308 in arm B, 124 were diagnosed HIV-infected by 12 weeks of age (transmission rate 1.88%; 95% CI: 1.56, 2.23). HIV infection was detected at birth, 6 weeks and 12 weeks in 64 (51.6%), 40 (32.3%), and 20 (16.1%) infants, respectively. Overall, ART was initiated in 116 (93.5%) infants within 2 days of diagnosis, including 35/38 (92.1%) infants diagnosed at birth. Proportions of infants virally suppressed at 24 weeks of age did not differ between arms, and were 7/30 (23.3%) in arm A and 6/22 (27.3%) in arm B with available viral load. After a median follow-up time of 23.9 weeks (IQR: 12.9, 26.3), 4 (5.8%) infants in arm A died at median 17.6 weeks, significantly lower than 8 (14.5%) in arm B at median 14.9 weeks (aHR 0.27; 95% CI: 0.08, 0.90; Figure 1).

Conclusion: PoC EID & treat at birth was feasible in resource-limited settings and resulted in a relative reduction of 73% in early mortality among HIV-infected infants. The combination of PoC EID at birth with dolutegravir-based infant ART might improve the poor viral suppression observed with LPI/ritonavir granules, potentially further enhancing the beneficial impact of birth test & treat on infant mortality.

Figure 1: Survival over time according to intervention group (A: PoC EID and ART initiation from birth; B: PoC EID and linkage to ART initiation at 4-6 weeks of age), with follow-up duration of 24 weeks of age (total n=124). Censored values (+) indicate last known follow-up time for infants still at risk.

133 POSTMORTEM CHARACTERIZATION OF HIV-ASSOCIATED UNDER-5 DEATHS IN FOUR CHAMPS SITES

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Background: The Child Health and Mortality Prevention Surveillance (CHAMPS) network conducts mortality surveillance in children using advanced postmortem techniques, providing a unique opportunity to investigate in depth causes of death (COD) in children in Africa and South Asia. Here we present CHAMPS COD findings in children who died from HIV (CDHIV) in four HIV high-burden countries.

Methods: CHAMPS collects comprehensive data on <5 years deaths in seven countries using minimally invasive tissue sampling (MITS), comprehensive pathogen screening using molecular methods, clinical record abstraction and verbal autopsy. Immediate, underlying, and comorbid COD are determined by a panel of multidisciplinary specialists using ICD-10 codes, after reviewing all available data. All 2016-2021 cases from Kenya, Mozambique, Sierra Leone and South Africa were included. Adjusted cause-specific mortality fractions (aCSMF) were calculated for HIV identified anywhere in the causal chain leading to death; aCSMF was defined as the proportion of deaths attributed to HIV among all cases completing expert review, adjusted using variables identified a priori from the literature and clinical expertise, including age, sex, location of death, season of death, and verbal autopsy cause of death

Results: Investigation of 1992 deaths from the four countries using MITS identified 106 (5.3%) with a confirmed HIV diagnosis in the causal chain; of which 6 (5.7%) were neonatal deaths, 46 (43.4%) were infant deaths, and 54 (50.9%) were in child (12-59 months) deaths. HIV aCSMFs were highest in Mozambique (9.68 [90% Bayesian CrI: 8.09–11.50] and Kenya (9.02 [90% Bayesian CrI: 6.60–12.03]), and lowest in Sierra Leone (4.14 [90% Bayesian CrI: 2.68–6.13]) and South Africa (3.90 [90% Bayesian CrI: 2.87–5.19]). Among the 106 CDHIV, 63 (56.7%) also had other respiratory infections, 42 (37.8%) had sepsis, 17 (15.3%) had malaria, 14 (12.6%) had diarrheal diseases, and 14 (12.6%) had other infections as COD (Figure 1). Kenya had higher prevalence of malaria in infants CDHIV than South Africa or Mozambique, while South Africa had more meningitis and liver disease than other sites.

Conclusion: HIV remains a major cause of child death in sub-Saharan Africa despite high coverage of prevention of mother-to-child-transmission (PMTCT) services. The most common other causes of death in HIV-infected children yield insight into interventions beyond pediatric ART that could have the greatest impact in reducing child deaths.
SARS-CoV-2 IN CHILDREN & ADOLESCENTS LIVING WITH HIV IN EUROPE & SOUTH AFRICA

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EPPICC (European Pregnancy and Paediatric Infections Cohort Collaboration)

Background: Adults living with HIV may have higher risk of SARS-CoV-2 infection than HIV negative adults. There are no published data on seroprevalence of SARS-CoV-2 in children and adolescents living with HIV (CALWHIV).

Methods: We did a repeat SARS-CoV-2 seroprevalence study in 7 paediatric HIV observational cohorts in 5 countries in the European Pregnancy & Paediatric Infections Cohort Collaboration (EPPICC, Belgium, Greece, Spain, Ukraine, United Kingdom (UK) and also the Cape Town Adolescent Antiretroviral Cohort (CTAAC), South Africa (SA) (CALWHIV and HIV negative adolescents). Participants gave 2 blood samples for SARS-CoV-2 antibody testing – 6 months apart during routine visits between May 2020 and July 2022, and completed questionnaires on SARS-CoV-2 exposure/infection and vaccine status. Clinical and demographic data were extracted from clinic records.

Results: Of 906 participants, 53% (477) were female, 89% (803) CALWHIV, median (IQR) age at first visit 17.15-19) years. Most were enrolled in SA (45%, 410/906), UK (23%, 205/906) or Ukraine (18%, 160/906). 85% (767/906) had 2 blood samples and the rest a single sample. For CALWHIV, at time of first sample, 99% (764/765) were on antiretroviral therapy, median CD4 count was 666 (478-858) cells/mL, 70% (535/764) had HIV-1 viral load < 50c/mL. Of those with known SARS-CoV-2 vaccine status, 23% (181/773) CALWHIV and 22% (22/100) HIV negative participants received ≥1 vaccine dose. 6% (43/763) of CALWHIV had a documented prior SARS-CoV-2 positive PCR (including 2 hospitalised for COVID, neither severe), and 16% (124/762) self-reported previous positive test and/or COVID-19 symptoms, giving a total of 17% (128/762) with any previous infection. Based on serum testing, 63% (562/898) of participants overall were seropositive on at least one sample (55% (269/488) Europe, 67% (205/307) SA CALWHIV, 85% (88/103) SA HIV negative group, and among the unvaccinated subgroup, 53% (408/765) were seropositive (41% (167/412) Europe, 64% (168/263) SA CALWHIV, 81% (73/90) SA HIV negative). Among samples taken prior to or in absence of vaccination, the proportion testing antibody positive increased over time (Figure). Of unvaccinated CALWHIV with ≥1 positive result, 17% (52/299) reported any previous SARS-CoV-2 infection.

Conclusion: Most CALWHIV were SARS-CoV-2 seropositive by mid-2022 despite low vaccine coverage. Few had documented or self-reported COVID-19 infection or disease, suggesting most infections were mild or asymptomatic.

SINGLE-CELL PROTEOGENOMIC PROFILING OF HIV-1 RESERVOIR CELLS


Background: HIV-1 reservoir cells persist lifelong despite ART but may be vulnerable to host immune responses that could be exploited for HIV-1 cure strategies. Here, we used a novel single-cell, next-generation sequencing approach for the direct ex vivo phenotypic profiling of individual HIV-1 infected memory CD4 (mCD4) T cells from peripheral blood (PB) and lymph nodes (LN) of people living with HIV-1.

Methods: mCD4 T cells derived from PB and LN of 8 participants were analyzed by a novel single-cell proteogenomic profiling assay. Cells were labeled with oligomer-tagged antibodies directed against n=53 surface markers and encapsulated into single cells. Multiplex PCR was conducted to amplify small fragments of genomic HIV-1 DNA (n=18) and the viral-host chromosomal junctions of large clones, coupled with amplification of antibody-bound oligomer tags. DNA and protein libraries were sequenced and biocomputationally deconvoluted.

Results: We analyzed n=530,143 individual mCD4 T cells from PB of 5 participants with around 10 years of suppressed viremia: n=2,859 represented infected HIV-infected cells; n=193 cells harbored genome-intact HIV-1; n=125 included clonally-expanded reservoir cells. Cells encoding for intact HIV or being part of large clones displayed more mature, effector memory phenotypic features and frequently expressed ensemble signatures of surface markers conferring increased resistance to immune-mediated killing (such as PVR and HVEM), paired with elevated expression of immune checkpoint markers (such as PD1 and KLRE1) likely to limit proviral gene transcription. A total of n=396,628 mCD4 T cells from LN were assayed from 3 participants who received ART for around 10-15 years: n=3,888 were HIV-1 infected cells, and n=111 cells harbored genome-intact HIV-1. Cells encoding for intact proviruses were equally distributed between T follicular helper cell (Tfh) and resident memory (TRM) CD4 cells and expressed higher levels of CD44, the IL-23 receptor (CD150), and the IL-7 receptor (CD127), all of which are associated with cell survival.

Conclusion: Cells encoding intact proviruses display distinct phenotypic features in PB and LN and upregulate markers associated with proviral transcriptional repression, resistance to immune-mediated killing, and cell survival. We propose that these phenotypic changes result from immune selection processes, implying that only small subsets of infected cells with optimal adaptation to their anatomical immune microenvironment can survive during long-term ART.
THE IMPACT OF 3BNC117, 10-1074, AND LEFITOLIMOD ON HIV-1 PERSISTENCE: THE TITAN TRIAL

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Background: Toll-like receptor 9 (TLR9) agonists stimulate innate antiviral immunity, prime adaptive immune responses and reverse latency in people with HIV-1 infection (PWH). Further, broadly neutralizing anti-HIV-1 antibodies (bNAb)s can boost HIV-specific immunity and enhance killing of infected cells through Fc-mediated mechanisms. Here, we evaluated the impact of the TLR9 agonist Lefitolimod and dual bNAbs treatment on ART-free virological control.

Methods: In a phase III multicenter, randomized placebo-controlled, double-blinded trial (TITAN study; NCT03837756), consenting PWH on suppressive ART were pre-screened for bNAbs sensitivity and randomized into one of four groups: A) placebo/placebo, B) lefitolimod (LEFI) /placebo; C) placebo/bNAbs; or D) LEFI/bNAbs. LEFI (120 mg) or placebo were given subcutaneously (SC) weekly from week 1 through week 8. 3BNC117 at 30 mg/kg and 10-1074 at 20 mg/kg or placebo were given intravenously (IV) at week 3 and at week 6. Analytical treatment interruption (ATI) of antiretroviral therapy went from week 3 to 26, or viral rebound, whichever came first. The primary endpoint was time to loss of virological control (plasma HIV-RNA > 1,000 c/mL for 4 weeks or confirmed >100,000 c/mL). Secondary endpoints were safety and rebound viral kinetics. bNAbs sensitivity was assessed by PhenoSense and HIV envelope sequencing.

Results: 46 participants were randomized to either placebo/placebo (n=11), LEFI/placebo (n=11), placebo/bNAbs (n=12), or LEFI/bNAbs (n=12). The median time to loss of virological control was 32 days (interquartile range [IQR] 21-77) in group A, 35 days (IQR 28-42) in group B, 119 days (IQR 77-175) in group C, and 98 days (IQR 70-119) in group D. The groups receiving bNAbs had significantly longer time to loss of virological control compared to placebo (log-rank, P = 0.0005). Six individuals did not meet ART restart criteria during the 24-week ATI (1 in B; 4 in C; 1 in D). The combination of LEFI and bNAbs vs. bNAbs alone did not result in improved virological control (log-rank, P = 0.42). More mild adverse events were reported in group D. Two severe adverse events were reported (one vasovagal reaction [unrelated] and one infusion related reaction [considered related to 3BNC117]).

Conclusion: In a double-blind trial among PWH on suppressive ART, harbouring virus sensitive to study bNAbs, the dual bNAbs treatment led to a significant delay in viral rebound during ATI, but there was no added effect on virological control of the TLR9 agonist, lefitolimod.
with Gag p55. Addition of anti-MHC class II antibody significantly reduced or prevented virus release, confirming that induction of HIV expression was dependent on interaction between peptide:MHCII–TCR. Without stimulation, CD4+ T cells didn’t lead to virus production. Conversely, non-specific activation with anti-CD3/CD28, when compared with autologous lysates, resulted in significantly higher virus production (HIV copies/mL, AUC 68,986 vs 2,352, p-value 0.0006) from multiple variants (average 5.71, range 1-12, versus 1-2 variants, respectively). Finally, in 3 of 5 participants with suppressed VL, stimulation with autologous lysates caused release of virus that matches QVOA isolates, suggesting this phenomenon is common among PLWH on ART, not just in those with NSV.

Conclusion: Our results show that recognition of self-antigens ex vivo can induce latency reversal and production of virus identical to infectious predominant plasma clones. This work provides a new understanding of spontaneous reservoir activity, explaining why only a rare subset of proviruses can cause persistent residual viremia and potentially viral rebound upon ART interruption.

139 **EX VIVO HIV DNA INTEGRATION IN STAT3 DRIVES T CELL PERSISTENCE AND OVERGROWTH**

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Background: Defective HIV proviruses within a few specific genes have been associated with T cell clonal expansion and persistence during ART, likely due to LTR-driven overexpression of the host gene via insertional mutagenesis. We have previously linked insertional mutagenesis of STAT3 to clonal expansion in T cells infected ex vivo with an HIV vector. Additionally, proviruses integrated in STAT3 and LCK have been implicated in insertional mutagenesis driving rare cases of AIDS-associated T cell lymphoma. We hypothesize that STAT3 integrations can lead to persistent HIV+ primary T cells infected ex vivo, which can model HIV-driven T- and possibly B- cell lymphoma.

Methods: Primary Human CD4+ T cells from three different donors were obtained from STEMCELL Technologies and infected with replication incompetent NL43dEnv with a gfp reporter. T cells were cultured in RPMI 1640 with IL-2 and stimulated with anti-CD3/CD28 beads. T cells from donors with persistent cells were taken for qPCR, Integration Site Analysis, and RNA-seq. T cells with persistent populations were later cultured with a Tat inhibitor (triptolide) to identify what role Tat and proviral LTRs plays in cell persistence. Flow cytometry was used to assess cell viability and gfp and STAT3 expression. Results: After day 126 post infection, three of the six replicates from one donor contained resurgent HIV (gfp) positive cell populations, with two of the three consisting of greater than 50% gfp+ cells. Integration site analysis identified the persistent HIV+ replicates all shared clonal integrations within Intron 1 of STAT3. RNA-seq identified overexpression of STAT3 and its downstream targets. These signatures were similar to HIV-driven T cell lymphomas described by Mellors et al, which shared similar STAT3 related expression patterns. The role of Tat in cell persistence needs to be further investigated but triptolide leads to a decrease in STAT3 expression and cell persistence.

Conclusion: We have confirmed that proviral integrations within STAT3 provide a selective advantage to primary CD4+ T cells infected with replication-incompetent HIV ex vivo. Additionally, these persistent HIV+ cells show similar overexpression patterns to HIV-driven T cell lymphomas thus providing a model for studying HIV-driven mutagenesis in T cells. We are exploring how Tat inhibition can reduce the selective advantage of proviral insertional mutagenesis, thus providing a potential therapeutic approach and prevention strategy for HIV-driven T cell lymphomas.

140 **THERAPEUTIC VACCINATION OF SHIV-INFECTED, ART-SUPPRESSED INFANT RHESUS MACAQUES**

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Background: Breastfeeding based HIV transmission accounts for the majority of new pediatric HIV infections. Antiretroviral therapy (ART) fails to eradicate HIV reservoirs, which lead to rapid viral rebound following ART interruption.

Therapeutic interventions effective in eliminating viral reservoirs and thereby delaying or preventing viral rebound in the absence of ART would be highly valuable for children living with HIV, who otherwise must be on ART for their lifetime. The objective of this study was to evaluate the immunological and virological impact of DNA/MVA/Protein-based therapeutic vaccination in a pediatric model of oral SHIV-infected ART-suppressed infant rhesus macaques (RMs).

Methods: A total of 19 infant RMs of ~4 wks of age were orally infected with SHIV.C.CS5S.375H.dC8 and placed on daily ART at 8 wpi. After complete viral suppression, a group of 9 animals was intramuscularly vaccinated with two doses each of SHIV-DNA + rCD40L, SHIV-MVA and CH69 SOSIP protein under ART. Ten RMs did not receive vaccinations and served as ART only controls. At 60 wpi, ART was interrupted (ATI) and all RMs were followed for 14 wks to study viral rebound kinetics. Peripheral blood (PB), lymph node (LN), and gut tissues were collected throughout the study for multiple virological and immunological analyses.

Results: DNA/MVA/Protein-based therapeutic vaccination was safe and highly effective in inducing highly functional SHIV-specific CD4+ and CD8+ T cell responses in PB and LN under ART. Vaccination also significantly expanded granzyme B- and perforin- expressing total and SHIV-specific CD8+ T cells in PB. HIV-Env specific binding and neutralizing antibody titers were increased significantly following vaccination and were significantly higher as compared to ART-only group. To our surprise, despite vaccine-induced robust immune activation, vaccination did not reduce viral reservoirs. Moreover, following ATI, no difference in terms of time to viral rebound or viral control were observed between the groups.

Conclusion: These data show that DNA/ MVA/Protein-based therapeutic vaccination is safe and effective in mounting robust SHIV-specific humoral and cellular immune response in SHIV-infected ART-treated infant RMs. Notably, additional interventions such as latency reversal agents or immune checkpoint blockade may be required to reduce viral reservoirs and alter rebound dynamics post ART release in vaccinated RMs.

141 **VIRAL RESERVOIR LANDSCAPE OF CHILDREN WITH HIV IN BOTSWANA TREATED WITH DUAL bNAbs**

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Background: To evaluate possible alternatives to ART among children living with HIV, the Tatelo clinical trial administered two broadly-neutralizing antibodies to children in Botswana with HIV clade C infection who had initiated ART at birth. Here we describe a high-resolution, cross-sectional and longitudinal analysis of the proviral reservoir landscape in Taleo participants.

Methods: Children were initially followed in the Early Infant Treatment (EIT) cohort, receiving continuous ART from birth; those entering Tatelo had HIV-1 RNA < 40 c/mL for ≥24 weeks prior to entry and were >96 wks of age. Tatelo participants received ART plus VRC01LS and 10-1074 (dosed every 4 wks) for at least 8 weeks, after which ART was stopped. Peripheral blood mononuclear cells were collected every 2-4 weeks. HIV-1 proviruses and chromosomal integration sites were analyzed using FLIP-seq and MIP-seq.
Results: Of 25 children treated with VR01-1S and 10-1074 alone, eleven (44%) maintained HIV RNA < 400 c/mL through 24 weeks (controllers) and 14 (56%) had viral rebound >400 c/mL (rebonders). In total, 592 proviral genomes (216 intact, 376 defective) were obtained; 31 integration sites of intact proviruses and 29 integration sites of defective proviruses were identified. Frequencies of total, intact and defective proviruses at birth were lower in controllers than in rebounders. No significant changes in intact, defective and total proviral frequencies were observed in controllers before and after bNAb treatment. Rebounders demonstrated a significantly higher reservoir of intact proviruses immediately prior to or at virological rebound compared to Tatelo entry (p=0.004). Proviruses were preferentially integrated into genes, and evidence for clonal expansion of proviruses with identical integration sites was detected in multiple children. In two controllers with high frequencies of viral reservoir cells, and one rebounder with low HIV RNA rebound (742 copies/mL), intact proviruses were preferentially (67%) integrated in centromeric/satellite DNA, ZNF genes and non-genic DNA.

Conclusion: In children with early initiation of ART, very low viral reservoirs were observed in controllers, without detectable differences in proviral reservoir size before and after bNAb treatment. In contrast, intact and defective proviruses were more commonly identifiable among rebounders beginning at birth, and increased in frequency between blnab initiation and rebound.

142 SINGLE-CELL EPIDENOMIC, TRANSCRIPTIONAL, AND PROTEIN STATES OF HIV RESERVOIR

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Background: The cellular program dictating productive HIV-1 infection versus latency remains unknown. Understanding the epigenetic regulators and transcriptional program of HIV-infected cells with undetectable HIV expression (HIV DNA+ RNA−) and active HIV expression (HIV RNA+) informs HIV eradication strategies.

Methods: We performed DOGMA-seq and TREK-seq to simultaneously capture 171 surface proteins, cellular transcriptome (RNA-seq), transcription factor (TF) activities (ATAC-seq), and T cell clonality (TCR sequencing) within the same single cells. We profiled paired memory CD4+ T cells during viremia and after suppressive ART from 6 HIV+ individuals from the Sabes cohort. Samples from 4 uninfected individuals served as controls.

Results: We captured 92,939 single cells (25,778 in viremia, 56,771 in viral suppression, and 10,600 in uninfected controls) having protein expression, transcriptomic, and epigenomic profiles. We identified 233 HIV DNA+ RNA− cells and 256 HIV RNA+ cells (38 HIV DNA+ RNA+ cells and 218 HIV DNA− RNA+ cells) in viremia and 20 HIV DNA+ RNA− cells and 14 HIV RNA+ cells (3 HIV DNA+ RNA+ cells and 11 HIV DNA− RNA+ cells) in viral suppression. Both HIV DNA+ cells and HIV RNA+ cells were clonally expanded in cytotoxic Th1 in both viremia and viral suppression. Epigenetic profiling revealed distinct increase in AP-1 TF activity during viremia in HIV RNA+ cells (q < 0.05) but not in HIV DNA+ RNA− cells in comparison to uninfected memory CD4+ T cells. Comparing HIV+ Th1 T cell clones between viremia and viral suppression, we found increased AP-1 TF activity in viremia (q < 0.05) and increased KLF and ZNF TF activities in viral suppression (q < 0.05). Transcription profiling revealed positive regulation of IFNγ production in HIV DNA+ cells (P < 0.005) and cytotoxic T cell proptotic process in HIV RNA+ cells (P < 0.005) in viremia. Compared to uninfected memory CD4+ T cells, we found 113 and 94 differentially expressed genes in HIV DNA+ RNA− cells and HIV RNA+ cells, respectively. Among them, IKZF3 and GZMA were significantly upregulated in both populations. In vitro validation showed that HIV-infected cells expressing IKZF3 had higher levels of cellular proliferation marker Ki67.

Conclusion: AP-1 transcription factor activity determines the high levels of HIV-1 expression in infected cells. IKZF3 may promote the proliferation of HIV-infected cells.

143 ANTI-IL-10/PD-1 IMMUNE MEDIATED CONTROL OF VIRAL REBOUND IN SIV INFECTED MACAQUES

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Background: IL10 and PD1 contribute to HIV reservoir persistence imped ing HIV eradication. IL10 contributes to lack of effector function, antigenpresentation and promotes HIV reservoir survival. PD1 leads to T cell exhaustion and HIV latency. We hypothesized that the dual blockade of IL10 and PD1 could synergize and lead to viral rebound control post-ATI (Analytical Treatment Interruption) by impeding on the survival of infected T cells and by rescuing T cell exhaustion and promoting memory and effector T-cell differentiation and function.

Methods: 28 rhesus macaques (RMs) infected with SIVmac239 started antiretroviral therapy (ART) 42 days post-infection which was maintained for 14-months. Rhesized all10 (n=10), all10 aPD1 (Combo therapy - n=10), or vehicle (n=8) was administered every 3-weeks starting 12 weeks pre-ATI and stopped 14-weeks post-ATI (week 26 PTX). Animals were followed longitudinally for biospecimen collections.

Results: Durable viral load control post-ATI (less than a 1000 cps/mL in 80% of the animals up to 6 months) in the combo treated RMs was associated with a biphasic immune response (Fig 1). I) pre-ATI poised immune response with i) significant induction of effector (GrzB+, Tbet+) and proliferating (Ki67+) CD8 T cells in LNs; ii) significant decay in the pro-survival protein BCL2 in CD4 T cells in LNs and PBMCs; and others and we have shown that BCL2 levels are higher in infected cells and iii) increased caspase mediates proinflammatory environment (IL1β, IL18, IP10, MIP3b and MCPs). These pre-ATI features were associated with control of viremia 24 weeks post ATI (week 36 PTX) and highlights a poised state of T cell differentiation providing advantage to the combo treated RMs as compared to all10 treated and control RMs.

Conclusion: The combo therapy set the stage for a poised immune effector state prior to ATI that enabled post-ATI differentiation and expansion of SIV specific CD4 and CD8 T cells leading to viral rebound control. A homeostatic environment, with preserved CD4 T cell counts and increased frequencies of cells expressing high levels of the stemness transcription factor TCF1, was uniquely observed in RMs that controlled the virus systemically and in tissues post-ATI. Fig 1. Graphical abstract. Phases of the immune response induced by the combo therapy leading to viral rebound control post-ATI.
144 EFFECTS OF EPLERENONE ON MYOCARDIAL PERFUSION AND FUNCTION: THE MIRACLE HIV STUDY

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Background: Subclinical cardiovascular disease (CVD) remains increased among persons with HIV (PWH). We have shown unique renin–angiotensin–aldosterone system (RAAS) physiology among PWH such that increased RAAS activation is associated with novel CVD risk factors, including visceral adiposity, insulin resistance, inflammation, and altered natriuretic peptides. We sought to understand how RAAS blockade may improve CVD in HIV.

Methods: 40 PWH without known CVD on stable ART with HIV viral load <100 c/mL were randomized to eplerenone 50mg (n=20) vs. placebo (n=20) BID in a 12-month double-blind, placebo-controlled trial. Myocardial indices were assessed by coronary PET and cardiac MRI. Change was determined by T-test or Wilcoxon Rank Sum Test depending on normality of distribution.

Results: Treatment groups (eplerenone vs. placebo) were similar for age (53.3±7 vs. 56.6±9 yrs), male sex (75% vs. 75%), white race (55% vs. 55%), HIV duration (20.8±8 vs. 21.7±9 yrs), and CD4+ count (814±617 vs. 740±543,1122 cells/mL). Change in coronary flow reserve (CFR) on coronary PET was not significantly different (0.01±0.04 vs. -0.07±0.04, P=NS, eplerenone vs. placebo). InITT analyses accounting for missing data, a higher proportion in the eplerenone vs. placebo group had improvements in CFR (75% vs. 40%, P=.02). In a subset analysis among those with impaired baseline CFR, eplerenone significantly improved CFR vs. placebo (0.28±0.27 vs. -0.05±0.36, P=.04). Eplerenone improved myocardial parameters on cardiac MRI including left ventricular end diastolic volume (13.28±2.10 vs. 10.2±2mL, P=.03), global circumferential strain (-1.31 vs. 2.0±4.1, P=.03), and stress myocardial blood flow (0.09±0.56 vs. -0.5±0.68 ml/min/g, P=.03). There was an increase in CD4+ count (2127-38 vs. -268-168, 53 cells/mL, P=.02) and a trend towards reductions in holl-6 (0.81±1.8, 0.4 vs. 0.2±0.5, 2.2±2pg/mL, P=0.07) and hs-CtF (0.01 vs. 0.00, 0.7±1L, P=.09) in the eplerenone vs. placebo-treated groups. Eplerenone was well-tolerated among PWH and lowered systolic blood pressure (-16±14 vs. -4±14 mmHg, P=.03) compared to placebo.

Conclusion: RAAS blockade with eplerenone improved key myocardial perfusion and diastolic dysfunction parameters among PWH. In addition, CFR improved significantly in a subset of PWH who had subclinical impairments in CFR at baseline. Moreover, eplerenone may have other novel properties to improve immune function in HIV, aside from its well-known mechanism of action related to BP and sodium regulation.

145 DEPRESSION AND ANXIETY ASSOCIATED WITH INCIDENT TYPE I AND II MI AMONG PWH

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Background: People with HIV (PWH) experience higher lifetime prevalence of major depression and anxiety than the general population. Depression and anxiety have been associated with increased risk of myocardial infarction (MI) in the general population and among PWH but with limited attention on the type of MI among PWH. We examined the association between depression and/or anxiety and incident Type 1 (TIMI) or Type 2 (T2MI) acute MI among PWH.

Methods: We examined data from 7 NA-ACCORD clinical cohorts (1997-2017) with adjudicated first MI, regardless of type; outcomes included TIMI (plaque rupture or cardiac intervention) or T2MI (demand ischemia). Baseline was when a participant entered observation for MI. We defined depression or anxiety as a time-varying ICD-coded diagnosis prior to an incident MI; we censored participants at death, loss to follow-up, or first MI (if it was not the outcome type of interest). We used Cox proportional hazard models to estimate the association between depression/anxiety and MI by type, adjusting for sex at birth, age, race/ethnicity, HIV acquisition group, substance use, and traditional and HIV-related risk factors for cardiovascular disease. We performed a test for interaction between depression and anxiety on the risk of MI.

Results: Of the 33,071 study participants followed for 168,846 person-years, 16,351 had a diagnosis of depression or anxiety (5,432 with both), and 16,720 never had a depression or anxiety diagnosis, 495 T1MIs and 374 T2MIs occurred. After adjusting for traditional and HIV-related risk factors, depression was a significant predictor of T1MI (aHR, 1.23 [95% CI, 1.02-1.49]) with a similar effect size and trend towards statistical significance for T2MI (aHR, 1.20 [95% CI, 0.96, 1.51]). Anxiety was not a significant predictor for T1MI (aHR, 0.92 [95% CI, 0.74-1.16]) but was significant for T2MI with a strong effect size (aHR, 1.42 [95% CI, 1.10-1.83]). We found no evidence for interaction between anxiety and depression. Ever smoking was a significant predictor for both T1MI and T2MI, while ever cocaine use and detectable viral load were significant predictors only for T2MI.

Conclusion: Diagnosed depression was a significant predictor of T1MI and suggestive for T2MI among PWH, whereas anxiety was associated only with T2MI. Further understanding of the role for mental health diagnosis and treatment to improve cardiovascular health in PWH is needed, including access to substance use disorder treatment and comorbidity management.

146 WEIGHT AND METABOLIC CHANGES WITH CABOTEGRAVIR+RILPIVIRINE LONG-ACTING OR BICTEGRAVIR

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Background: Integrase strand transfer inhibitors (InSTIs) and tenofovir alafenamide–based regimens are potentially associated with weight gain and metabolic perturbations in people living with HIV (PWH). Cabotegravir (CAB), an InSTI, plus rilpivirine (RPV), a non-nucleoside reverse transcriptase inhibitor, administered monthly or every 2 months (Q2M) is the first complete long-acting (LA) regimen recommended by treatment guidelines for the maintenance of HIV-1 virologic suppression. Modest body weight and lipid changes have been observed in participants receiving CAB+RPV LA therapy in Phase 3/3b studies. SOLAR (NCT04542070) is a Phase 3b noninferiority efficacy study, also evaluating weight and metabolic changes from baseline (BL) to Month (M)11/12 as additional endpoints, in PLWH switching to CAB+RPV LA therapy vs. continuing on bictegravir/emtricitabine/tenofovir alafenamide (B/FTC/TAF).

Methods: Among 687 participants randomized (2:1; n=6 not dosed), 454 switched to CAB+RPV LA Q2M (175 elected for oral lead-in [OLI] and 279 elected without an OLI) and 227 continued on B/FTC/TAF. Changes from BL to Month 11 and Month 12 were compared to Month 0 among participants randomized to CAB+RPV LA Q2M (OLI) and B/FTC/TAF as additional endpoints, in PLWH switching to CAB+RPV LA therapy vs. continuing on B/FTC/TAF. Changes from BL and Month 0 were compared to Month 0 among participants randomized to CAB+RPV LA Q2M (OLI) and B/FTC/TAF as additional endpoints, in PLWH switching to CAB+RPV LA therapy vs. continuing on B/FTC/TAF.

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in body weight, body mass index (BMI) category, waist and hip circumferences (WC, HC), waist-to-height ratio (WHtR), waist-to-hip ratio (WHR), muscle mass, total body fat, and proportion of participants with insulin resistance or metabolic syndrome were analyzed at M11 (LA without OLI)/M12 (LA with OLI and B/FTC/TAF).

**Results:** Median (interquartile range) change in body weight from BL was $-0.40 \text{ kg} (-2.95, 2.10)$ in the LA arm and $+0.05 \text{ kg} (-2.30, 1.95)$ in the B/FTC/TAF arm at M11/12 (Table). Mean (standard deviation) change in WC and HC was $+0.19 \text{ cm} (8.01)$ and $+0.26 \text{ cm} (7.81)$ in the LA arm, and $+1.64 \text{ cm} (9.19)$ and $+0.51 \text{ cm} (11.44)$ in the B/FTC/TAF arm at M11/12. There were no clinically relevant changes in BMI or WHR, or the proportion of participants with metabolic syndrome, abdominal obesity, or insulin resistance in either arm.

**Conclusion:** This is the first randomized Phase 3b study to compare weight, anthropometric, and metabolic changes in a standardized manner among PLWH switching to CAB+RPV LA Q2M or continuing B/FTC/TAF. Changes in weight, BMI, and body composition measurements were minor and similar between treatment arms through M11/M12. There were no clinically relevant changes in the proportion of participants with metabolic syndrome, abdominal obesity, or insulin resistance between arms at M11/12.

### Baseline Characteristics, Weight, and Metabolic Changes Through Month 11 or Month 12 From SOLAR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CAB+RPV LA</th>
<th>B/FTC/TAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>57 (47, 66)</td>
<td>56 (46, 69)</td>
</tr>
<tr>
<td>Median BMI (kg/m²)</td>
<td>24.5 (22.8, 26.2)</td>
<td>24.3 (22.5, 26.1)</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>37.0 (30.0, 44.0)</td>
<td>37.5 (30.0, 44.0)</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>31.7%</td>
<td>27.5%</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>36.8%</td>
<td>32.7%</td>
</tr>
</tbody>
</table>

**Results:** Median (interquartile range) change in body weight from BL was $-0.40 \text{ kg} (-2.95, 2.10)$ in the LA arm and $+0.05 \text{ kg} (-2.30, 1.95)$ in the B/FTC/TAF arm at M11/12 (Table). Mean (standard deviation) change in WC and HC was $+0.19 \text{ cm} (8.01)$ and $+0.26 \text{ cm} (7.81)$ in the LA arm, and $+1.64 \text{ cm} (9.19)$ and $+0.51 \text{ cm} (11.44)$ in the B/FTC/TAF arm at M11/12. There were no clinically relevant changes in BMI or WHR, or the proportion of participants with metabolic syndrome, abdominal obesity, or insulin resistance in either arm.

**Conclusion:** This is the first randomized Phase 3b study to compare weight, anthropometric, and metabolic changes in a standardized manner among PLWH switching to CAB+RPV LA Q2M or continuing B/FTC/TAF. Changes in weight, BMI, and body composition measurements were minor and similar between treatment arms through M11/M12. There were no clinically relevant changes in the proportion of participants with metabolic syndrome, abdominal obesity, or insulin resistance between arms at M11/12.

### 147 A LOSS OF ERα ATTENUATES DTG-MEDIATED DISRUPTION OF THERMOGENESIS IN BROWN ADIPOCYTES

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**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 of sex-difference are unclear. Estrogen is recognized as a master regulator of the energy homeostasis and is a major determinant of sex differences in the control of energy homeostasis. Hence, we hypothesized that InSTIs may interrupt adipose function via estrogen receptor.

**Methods:** Estrogen receptor-mediated transcriptional activity was measured by luciferase reporter containing estrogen response element in the presence of dolutegravir (DTG), doravirine (DOR) and efavirenz (EFV). Estrogen receptor α (ERα) deficient cells were created from primary preadipocytes isolated from female mice (ERα loxp/loxp) using a Cre/loxp system. Cells were treated with DTG for 8 days during differentiation into mature white or brown adipocytes. Mature adipocytes were analyzed for lipid accumulation by Oil Red O staining, adipogenic markers by qRT-PCR and immunoblotting.

**Results:** We found that DTG treatment reduced estrogen-mediated activation in a dose dependent manner and half of Cmax value of DTG (2.5 uM) was sufficient to completely suppress reporter activity. However, DOR and EFV had no effect. We found that a genetic deletion of ERα by Ad-Cre alone did not affect overall adipogenesis as adipogenic markers (PPARγ, FABP4, and CEBPα/β) levels and lipid accumulation by Oil Red O staining were similar between ERα knockout (KO) and wild type (WT) adipocytes. However, a loss of ERα significantly attenuated DTG-mediated suppression of uncoupling protein 1 (UCP1), which is an essential for a thermogenic process in brown/beige adipocytes (Figure), and other brown adipogenesis markers (Dio2, CIDEA) in ERα KO compared to WT adipocytes. In addition, DTG-mediated suppression of mitochondrial respiratory chain, cytochrome oxidase complex IV (COX IV), lipid droplet associated protein 1 (perilipin1), hormone sensitive lipase (HSL) was blocked in ERα KO compared to WT adipocytes.

**Conclusion:** Our data showed that DTG inhibits estrogen signaling action modulated by ERα and a genetic deletion of ERα in adipocytes attenuates DTG-mediated suppression of thermogenesis. These findings suggest a novel
MECHANISM BY WHICH INSTIS MAY LEAD TO WEIGHT GAIN POTENTIALLY IN A SEX-DEPENDENT MANNER.

Figure A. A loss of ERα attenuates DTG effect on UCP1 expression in brown adipocytes (n=6, *, P<0.05).

148 MICROBIOTA-DERIVED METABOLITES ARE POWERFUL BIOMARKERS FOR ANAL CANCER PREVENTION

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Background: The risk of anal cancer is markedly increased in PWH, especially in MSM, who exhibit specific microbiota signatures, potentially contributing to the greater HPV persistence and oncogenic potential. The low specificity of the current screening strategy for detecting high-grade squamous intraepithelial lesions (HSIL) hinders anal cancer prevention. We investigated in anal cytology samples microbiota derivatives associated with HSIL.

Methods: We recruited a discovery and a validation cohort in 4 clinical sites in Spain and Italy. Study participants were mostly MSM undergoing HSIL screening with high-resolution anoscopy and anal biopsies to confirm HSIL. We extracted the bacterial DNA, proteins, and metabolites from anal cytology samples, where we performed 16S RNA gene sequencing, mass spectrometry, and targeted metabolite quantification.

Results: We included 213 participants, 167 in the discovery cohort (70 with confirmed HSIL), and 46 in the validation cohort (25 with confirmed HSIL). Patients with HSIL exhibited an increased abundance of Prevotella copri, while Sneathia sanguinegens were depleted. The microbiota associated with HSIL overexpressed proteins that converged in the production of succinyl-CoA and cobalamin, which levels were consistently increased in subjects with HSIL (Figure 1A).

The combination of succinyl-CoA and cobalamin overperformed the anal cytology, improving sensitivity from 91.2% to 96.6%, specificity from 34.1% to 81.8%, positive predictive value from 48.1% to 77.8%, and negative predictive value from 83.3% to 97.3% (Figure 1B). While the anal cytology correctly classified only 59.9% of individuals, the combination of both metabolites improved the classification to 87.7%. This test overcame internal (adjusted AUC 0.877) and external validation. From 98 false-positive cytologic results we reclassified to true negative results 49 (81.8%, positive predictive value from 48.1% to 77.8%, and negative predictive value from 83.3% to 97.3%)

Conclusion: Cobalamin and succinyl-CoA are overexpressed in the anal microbiota of patients with HSIL and show an excellent diagnostic capacity. Their measurement overperforms anal cytology to screen for patients with HSIL. Hence, we discovered two powerful biomarkers, for which readily detection methods can be established, that could improve the current strategy for anal cancer screening.

149 IMPACT OF INTEGRASE INHIBITORS ON CARDIOVASCULAR EVENTS IN PERSONS STARTING ART

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Background: Integrase strand transfer inhibitors (INSTIs) have become essential components of antiretroviral therapy (ART). A recent study suggests that INSTI-based ART may lead to an increased risk for cardiovascular (CVD) events in the first two years compared to other ART combinations. However, analyzing treatment-naïve and treatment-experienced individuals together may have introduced bias. We estimated the impact of starting INSTI-based compared to other ART on cardiovascular events among treatment-naive people with HIV (PWH) by emulating a target trial to minimize the risk of bias.

Methods: We included participants from the Swiss HIV Cohort Study who were treatment-naïve after May 2008, when the first INSTI became available in Switzerland. Baseline was defined as the date of the first treatment start (INSTI vs. other ART), and individuals were followed until the first CVD event (myocardial infarction, stroke, or intervention on arteries), loss to follow-up, death, or last cohort visit. Individuals who stopped the initial strategy (INSTI or other ART) were censored at that time. We estimated cardiovascular disease risk differences using pooled logistic regression with inverse probability of treatment and censoring weights, taking into account time-fixed and time-varying confounders (covariates listed in figure legend).

Results: Of 5287 treatment-naïve individuals, 2032 (38.4%) started INSTI-based ART, and 3255 (61.6%) started other ART combinations. Individuals who started INSTI were less likely to be women (16% vs. 26%), less likely to be of African origin (12% vs. 19%), and had a higher median CD4 nadir (346 vs. 281 cells/µL) compared to individuals starting other ART. Smoking status, history of cardiovascular disease, and use of antplatelet agents were similar in both groups, but INSTI-starters were more likely to receive abacavir (26% vs. 11%). Within 25’567 person-years (PY), 113 CVD events occurred (incidence rate 4.42 per 1000 PY, 95% CI 3.68–5.31). Unadjusted incidence rates were 6.49 per 1000 PY (4.99–8.46) among INSTI starters and 3.39 per 1000 PY (2.62–4.39) among those who started other ART (Panel A). In adjusted analyses, risk differences between INSTI and other ART starters were -0.02% (-0.32% to 0.21%) after 1 year, -0.17% (-0.65% to 0.30%) after 2 years, and -0.38% (-1.29 to 0.52) after 5 years (Panel B).

Conclusion: In this target trial emulation, we found no evidence of a difference in CVD event risk with starting INSTI-based compared to other ART in treatment-naive PWH.
Cardiovascular disease events in people living with HIV starting ART
Panel A shows unadjusted Kaplan-Meier curves. Panel B shows survival curves adjusted for calendar year of ART start, sex, age, ethnicity, HIV transmission risk group, education level, nadir CD4, history of AIDS-defining disease, history of cardiovascular disease, family history of cardiovascular disease, hepatitis B and C virus coinfection, arterial hypertension, diabetes, renal function, and time-varying smoking status, use of abacavir, tenofovir alafenamide, antiputeptide and lipid-lowering drugs. Shaded area = 95% confidence interval. ART = antiretroviral therapy. CVD = cardiovascular disease. INSTI = integrase inhibitors.

150 HIV-RELATED KAPOSI SARCOMA IN EAST AFRICA: CONTEMPORARY UPDATE ON STAGE AND SURVIVAL
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Background: Stage at time of diagnosis and survival after diagnosis are critical parameters regarding the control of any cancer in any geographical setting. Unlike in resource-rich settings where publicly funded cancer surveillance routinely monitors these parameters, these data are non-existent through routine means in resource-limited areas. This is particularly relevant for Kaposi sarcoma (KS) in East Africa, for which recent changes in HIV treatment and chemotherapy guidelines as well as the COVID-19 pandemic dictate an update regarding stage and survival.

Methods: From October 2021 to August 2022, we evaluated HIV-infected adults (age ≥ 18 years) with a new diagnosis of KS made in 4 different primary care facilities (or their associated inpatient units) in Kenya and Uganda using a process of rapid case ascertainment. KS diagnosis was confirmed by pathology. Participants were examined, at time of biopsy, to document the extent of lesions and subsequently monitored longitudinally for vital status.

Results: Among 180 HIV-infected adults identified with new onset KS, 31% were women, and the median (IQR) age was 35 (29–42) years. At time of KS diagnosis, 95% of the participants were taking ART, and the median (IQR) CD4+ T cell count was 197 (46-354) cells/mm³; 46%, 20%, 11% and 23% had plasma HIV RNA of < 40, 40-1000, 1001-10,000 and > 10,000 copies/ml, respectively.

Conclusion: In a recently assembled community-based sample of adults with newly-diagnosed HIV-related KS in East Africa, the majority have advanced KS at the time of KS diagnosis, and survival is poor. The findings are stark in absolute terms for the “Treat-All” era and unchanged from parameters obtained in the 5 years prior, indicating no improvement in these aspects of the control of KS in the region. Along with primary prevention of KS (i.e., reducing its incidence), novel approaches are needed for earlier detection, more efficient linkage to oncologic care, and more potent therapy.

Survival Among Adults with HIV-Related Kaposi Sarcoma in East Africa

151 PHYLODYNAMICS OF HIV-1 VARIANT ACROSS POLAND AND UKRAINE
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Background: In the countries of Eastern Europe (Russia, Belarus, Ukraine), where the HIV-1 epidemics are expanding, the A6 variant remains predominant. After the initiation of ART in Ukraine, Poland has become the principal refuge for Ukrainian citizens; however, migration from this country has been common over the last decade. Evolutionary analyses of viral sequences provide insights into migration events. The aim of this study was to explore the transmission dynamics of the HIV-1 A6 variant between Poland and Ukraine.

Methods: HIV A6 partial pol sequences from Poland (n=1185) and Ukraine (n=653) were combined with a background dataset of 7665 publicly available sequences from 37 other countries. We used maximum-likelihood phylogenetic and Bayesian inference to identify putative transmission clades. Clades were analyzed in an asymmetric discrete phylogeographic model to characterize HIV migration events (adjusted Bayes factor ≥3) between administrative regions of Poland and Ukraine.

Results: We identified 206 clades (n=1,362 sequences), of these, 63 were binational, containing both Polish and Ukrainian sequences. Migration events were almost exclusively unidirectional from Ukraine to Poland (99.4%). Multiple cross-border migrations were mainly from East and South Ukraine to Central and Western Poland. Donetsk and Odessa regions were the primary source (49.7% and 17.6%, respectively) of the variant dispersal. The most frequent destinations were in Masovian, the highest populated region where Warsaw, the largest city and also the capital of Poland, is located (67.3%); next in Lodz (18.2%); and West Pomerania (10.1%) states. Migration events started to increase in 2011 and remained stable until 2016, at the time of the initial war in the Donetsk region.

Conclusion: The most common HIV-1 A6 variant migration events to Poland were from Eastern Ukraine long time affected by the Russian-Ukrainian armed conflict. Geospatial A6 transmission networks are expanding in Poland, sustained by local viral dispersal and cross-border migration originating from Ukraine. More introductions are expected since the initiation of war in 2022, which may notably increase the burden of HIV in Central and Western European countries. There is an urgent need for expanded HIV diagnostics and antiretroviral treatment initiations among war-displaced populations to reduce the transmission networks.

Sampling density and phylodynamics of HIV A6 variant migration events to Poland and Ukraine A. Sampling density of HIV-1 A6 variant. Regions with a lack of sequences were depicted in grey; Migration events between regions in Poland and Ukraine through time B. Between 2011 and 2013; C. Between 2014 and 2016. The thickness of the arrows corresponds to the average number of inferred migration events. Inward movements between a particular group and location are depicted in the same color. Regions are colored according to the number of sequences included in the clade. Phylogeographic inference was performed using the asymmetric discrete phylogeographic model implemented in the BEAST 1.10.5 software package.
**152 TIME TO KEY MILESTONES IN THE HIV INDEX TESTING/CONTACT TRACING PATHWAY IN INDIA**

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**Background:** Index testing, a WHO-endorsed form of contact tracing, is an evidence-based approach to enhance detection of people living with HIV (PLHIV). To optimize impact and efficiency, the process should rapidly identify contacts of newly diagnosed PLHIV and link them to immediate treatment. We estimate the time taken (in days) for each milestone along the index testing pathway from contact elicitation to linking HIV-positive contacts to treatment in one of the highest HIV burden states in India, Telangana.

**Methods:** As part of a PEPFAR-funded program, we implemented index testing in 50 testing and treatment facilities in five districts of Telangana. We analyzed program data from July 2020-January 2022. We calculated time (same day, within a week, > a week) between steps along the index testing continuum defined as the date when: 1) contact elicitation was started; 2) contact was notified; 3) contact was tested for HIV; 4) contact received results; and 5) HIV positive contact was linked to treatment. Associations between contact characteristics and time category were evaluated using chi-square tests.

**Results:** In total, 15,253 contacts were elicited and notified, 13,335 were tested within a week. > a week) between steps along the index testing continuum (interquartile range, 17-92) with 24% eventually testing HIV positive (compared to 15% positivity in spousal contacts who took > a week). Once HIV testing was complete, the experience of sexual partners was comparable to spousal contacts. Results were received the same day and most were linked to treatment within a week.

**Conclusion:** Overall, index testing was an efficient way to identify contacts and initiate newly diagnosed PLHIV on antiretroviral therapy. However, these data highlight challenges in identifying vulnerable sexual partners and the need for differentiated approaches to avoid delays and curtail ongoing HIV transmission. Figure - Time Between Index Testing Milestones by Type of Contact Elicited

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**153 ASSISTED PARTNER SERVICES FOR PWID TO IDENTIFY PARTNERS LIVING WITH HIV & HCV**

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**Background:** People who inject drugs (PWID) experience high risk of both HIV and hepatitis C (HCV) but face barriers to testing and engagement in care. Assisted partner services (APS) is effective at case finding for HIV but has been understudied among PWID. We determined whether APS could find, test for HIV and HCV, and link to care the sexual and injection partners of PWID living with HIV in Kenya.

**Methods:** We conducted a prospective study in 8 sites in Kenya. Index participants—people living with HIV (PLHIV) actively injecting drugs—were interviewed and provided locator information about sexual and injection partners. Peer educators located partners and brought them to study sites, where partners completed questionnaires and rapid HIV testing. All participants also had rapid HCV antibody testing and all participants positive for HIV or HCV antibodies underwent a blood draw for viral load testing. Intimate partner violence (IPV) was monitored for all participants at moderate or high risk. Data were entered into REDCap and analyzed using Stata. Numbers needed to interview (NNTI) to find partners in need of care were calculated.

**Results:** 989 index participants mentioned 4705 partners and 4588 (97%) of mentioned partners were located, of whom 18.0% were living with HIV and 11.8% were HCV antibody-positive. Of the 597 living with HIV, 85.2% already knew their status, of whom 91.3% reported currently taking ART and 77% were virally suppressed. NNTIs are shown in Table 1. Of 393 partners positive for HCV antibodies, 53.7% were viemic, 26.5% already knew their antibody status, and 1% had previously been treated. Prevalence of both HIV and HCV differed by partner type. 6-month follow-up completion was 86%. Over 70% of index participants and partners who were not on antiretroviral therapy at enrollment were taking it at 6-month follow-up visits. No IPV or adverse events, including violence or death, occurred due to study procedures.

**Conclusion:** When used among PWID, APS for PLHIV is safe and effectively identifies partners living with both HIV and HCV. Although many partners were already aware of their HIV status, APS also increased engagement in care for both index participants and partners over a 6-month period.

**Table 1. Numbers of index participants interviewed to find partners in different categories**

<table>
<thead>
<tr>
<th>Category</th>
<th>Index Participants</th>
<th>NNTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Partners</td>
<td>989</td>
<td>4705</td>
</tr>
<tr>
<td>A partner living with HIV (PLHIV)</td>
<td>473</td>
<td>1.66</td>
</tr>
<tr>
<td>A partner living with HCV</td>
<td>473</td>
<td>3.68</td>
</tr>
<tr>
<td>A partner living with both HIV and HCV</td>
<td>473</td>
<td>6.08</td>
</tr>
<tr>
<td>Injecting Partners</td>
<td>439</td>
<td>2.87</td>
</tr>
<tr>
<td>A partner living with HIV (PLHIV)</td>
<td>439</td>
<td>1.72</td>
</tr>
<tr>
<td>A partner living with HCV</td>
<td>439</td>
<td>3.60</td>
</tr>
<tr>
<td>A partner living with both HIV and HCV</td>
<td>439</td>
<td>5.85</td>
</tr>
<tr>
<td>Sexual Partners</td>
<td>546</td>
<td>8.17</td>
</tr>
<tr>
<td>A partner living with HIV (PLHIV)</td>
<td>546</td>
<td>1.66</td>
</tr>
<tr>
<td>A partner living with HCV</td>
<td>546</td>
<td>3.68</td>
</tr>
<tr>
<td>A partner living with both HIV and HCV</td>
<td>546</td>
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154 HIV INCIDENCE DIFFERENCES MEASURED BY SERIAL CROSS-SECTIONAL TESTING: HPTN 071
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Background: HPTN 071 (PopART) was a community-randomized trial conducted in Zambia and South Africa (2013-2018); 21 communities were randomized into three arms in 7 triplets matched on baseline HIV prevalence and location (Arm A: combination prevention intervention with universal antiretroviral treatment [ART]; Arm B: combination prevention intervention with ART provided according to local guidelines; Arm C: standard of care). In the primary analysis, intervention effect was assessed using longitudinal observed HIV seroconversion. Arm B had 30% lower incidence than Arm C, in contrast, the intervention effect was similar in Arms A and C. In this report, we used cross-sectional incidence (CSI) testing to estimate HIV incidence in each community at baseline (PC0) and two-year visit (PC24).

Methods: CSI was estimated using the LAg assay and HIV viral load (mean duration of recent infection: 130 days; false recent rate: 0.2%). This method was shown to accurately estimate incidence in this cohort. The Sedia Incidence Calculator was used to calculate incidence estimates. Intervention effect compared CSI at PC24 between arms, adjusted for community age and sex composition and PCI CSI.

Results: At PC0, 37,128 individuals were surveyed; 7,998 were HIV+ and 187 were classified as recently infected. At PC24, 27,896 individuals were surveyed; 6,254 were HIV+ and 73 were classified as recently infected. CSI testing was performed for 99.5% of all HIV+ samples. HIV incidence at enrollment was highest in Arm A in 4 of 7 triplets. The unadjusted incidence estimates for Arms A, B and C were 1.95, 1.49 and 1.58 at PC0 and 1.03, 0.90 and 1.55 /100py at PC24, respectively. Based on CSI, the adjusted HIV incidence ratio for Arm A vs. C was 0.69 (95% CI: 0.44-1.07, p=0.095) and for Arm B vs. C was 0.54 (95% CI: 0.34-0.84, p=0.011).

Conclusion: In this large community-randomized trial, HIV incidence decreased over time in both study arms that included a combination prevention intervention. The decrease in incidence was similar in communities with universal ART (Arm A, 33% reduction) and ART provided according to local guidelines (Arm B, 46% reduction). The methods used for the intervention assessment using CSI align with those used for the primary study analysis and suggest that intervention was similarly effective in both intervention arms. Additionally, the apparent dissonance in the effects in Arms A and B (in the primary analysis) may have been due to a chance imbalance in HIV incidence at baseline.

155 INCIDENCE OF CANCER AMONG MEDICAID BENEFICIARIES WITH AND WITHOUT HIV: 2001-2015
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Background: Life expectancy among people living with HIV (PLH) is approaching the general population, and chronic conditions associated with aging—including cancer—are increasingly relevant. Among PLH, incidence of AIDS-defining cancers (ADCs) has decreased, and the cancer burden has shifted toward non-AIDS-defining cancers (NADCs). Medicaid provides insurance for 40% of PLH in the US and may offer a better comparator group for PLH than the general population. Thus, we compared the incidence of cancer among Medicaid beneficiaries with and without HIV.

Methods: We used data on Medicaid beneficiaries enrolled in 14 US states in 2001-2015. We included beneficiaries aged 18-64 years, who had >6 months of continual enrollment in Medicaid without dual enrollment in Medicare. We excluded beneficiaries with a history of cancer prior to baseline. We estimated cumulative incidence of first cancer by HIV status at baseline, with age as the time scale, accounting for death and incidence of other cancer types as competing risks. Cancer types examined included all ADCs (Kaposi’s sarcoma, cervical, and non-Hodgkin’s lymphoma) and NADCs that are high burden in the general population (breast, colon, head/neck, kidney, larynx, leukemia, liver, lung, oropharynx, pancreatic, prostate) and among PLH (anal, Hodgkin’s lymphoma). We estimated cumulative incidence overall and stratified by sex, race/ethnicity, and calendar period.

Results: We included 43,925,817 beneficiaries in the analysis, of whom 181,030 had HIV. Across all ages, beneficiaries with HIV had a higher incidence of ADCs, as well as some NADCs (lung, leukemia, head and neck, liver, oropharynx, larynx, and anal cancer). Breast cancer incidence was similar until age 42, after which beneficiaries without HIV had a higher incidence. For colon, prostate, and pancreatic cancers, beneficiaries with HIV had higher incidence at younger ages; at older ages, incidence was similar or higher among beneficiaries without HIV.

Conclusion: We compared cancer incidence by HIV status within a geographically diverse sample of Medicaid beneficiaries. We examined incidence across much of the beneficiaries’ adult lives, allowing us to see trends in how incidence of cancers (especially NADCs) varied by HIV status and age. Our findings may suggest an increased risk of certain NADCs (e.g., colon, head/neck, and leukemia) at younger ages among PLH, even when compared against other Medicaid beneficiaries, and highlight the importance of monitoring PLH for both ADCs and NADCs.

Incidence of AIDS-defining cancers, non-AIDS-defining cancers, and death by HIV status in all eligible Medicaid beneficiaries, 2001-2015

156 CAUSES OF DEATH AMONG ADULTS WITH HIV IN EUROPE AND NORTH AMERICA: 1996-2019
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Background: Mortality rates among persons with HIV (PWH) have fallen since widespread availability and use of effective antiretroviral therapy (ART) in 1996. Patterns of cause-specific mortality continue to change as the population of PWH ages.

Methods: We investigated longitudinal patterns in causes of death in Europe and North America among PWH aged ≥16 years old who started ART 1996-2019, using data from the Antiretroviral Therapy Cohort Collaboration. Cohorts gathered information on mortality through hospitals or physician reports, linkage with vital statistics agencies and active follow-up. Causes of death were coded using adaptation of the Coding of Death in HIV protocol. Causes were mostly classified by both a clinician and an algorithm using available ICD9/10 data on cause of death and otherwise two clinicians using clinical data with
all disagreements being resolved through panel discussion. We used Poisson models to compare cause-specific mortality rates over time-periods, adjusted for time-updated CD4, age, and whether ART-naïve at the start of each follow-up period.

**Results:** Among 189,916 PWI, 16,897 died. Age at death increased from 42.2 years in 1996-99 to 56.2 years in 2016-19. Rates of all-cause mortality per 1000 person-years decreased from 16.9 (95% confidence interval [95% CI]: 15.4-18.4) during 1996-99, to 7.9 (7.6-8.2) during 2016-20. The adjusted all-cause mortality rate ratio (aMRR) per 4-year calendar period was 0.86 (95% CI 0.85-0.87), see table. Mortality rates declined with time for almost all causes of death. The decline over time was most pronounced for AIDS-related mortality (aMRR per 4 years 0.82; 95% CI 0.80-0.84). There were also reductions in cardiovascular, liver-related, non-AIDS infection, non-AIDS, non-hepaticellular carcinoma malignancy, and suicide/accident mortality (corresponding aMRRs per 4 years 0.83 (0.79-0.87), 0.90 (0.85-0.94), 0.93 (0.88-0.98), 0.95 (0.91-0.98), and 0.90 (0.84-0.96), respectively). There was little evidence of declines in central nervous system, respiratory, and substance-use related mortality.

**Conclusion:** Improvements in ART and HIV care have led to reductions in rates of all major causes of death, particularly AIDS-related deaths, among PWI taking ART. These results highlight the further potential for reducing mortality through hepatitis treatment, targeted cancer screening, and preventing late HIV diagnosis. Adjusted cause-specific mortality rate ratios (MRRs) per 4-year period.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Deaths</th>
<th>MRR per 4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>16897</td>
<td>0.86 (0.84, 0.87)</td>
</tr>
<tr>
<td>AIDS</td>
<td>4222</td>
<td>0.82 (0.80, 0.84)</td>
</tr>
<tr>
<td>Cardiovascular/heart (including stroke)</td>
<td>1406</td>
<td>0.83 (0.79, 0.87)</td>
</tr>
<tr>
<td>Central nervous system (including Parkinson’s and Alzheimer’s)</td>
<td>184</td>
<td>1.02 (0.94, 1.04)</td>
</tr>
<tr>
<td>Liver (including hepaticellular carcinoma)</td>
<td>1567</td>
<td>0.80 (0.74, 0.86)</td>
</tr>
<tr>
<td>Non-AIDS infection</td>
<td>1048</td>
<td>0.93 (0.88, 0.98)</td>
</tr>
<tr>
<td>Non-AIDS, non-hepaticellular carcinoma malignancy</td>
<td>3314</td>
<td>0.79 (0.71, 0.88)</td>
</tr>
<tr>
<td>Other</td>
<td>1461</td>
<td>0.80 (0.73, 0.86)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>262</td>
<td>1.00 (0.96, 1.03)</td>
</tr>
<tr>
<td>Substance abuse</td>
<td>380</td>
<td>0.94 (0.86, 1.03)</td>
</tr>
<tr>
<td>Suicide/accident</td>
<td>603</td>
<td>0.80 (0.74, 0.86)</td>
</tr>
<tr>
<td>Unknown/undescribable</td>
<td>3082</td>
<td>0.83 (0.81, 0.85)</td>
</tr>
</tbody>
</table>

157  **MOLECULAR HIV CLUSTERING OF INDIVIDUALS WITH MPOX/HIV COMORBIDITY**

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**Background:** HIV cluster detection and response (CDR) is a key part of the federal Ending the HIV Epidemic initiative in the United States. HIV molecular clusters are thought to preferentially include individuals that are at highest risk of HIV transmission and the most stringent clusters are assumed to indicate recent and rapid transmission. The global monkeypox (MPOX) outbreak, starting in 2022, created an opportunity to test the utility of molecular HIV surveillance (MHS) to identify high risk transmission networks.

**Methods:** In New York State (NYS) outside New York City (Rest of State - ROS) there were 282 individuals diagnosed with MPOX from June 2, 2022 through September 9, 2022. Individuals diagnosed with MPOX were matched to the NYS HIV and sexually transmitted infection (STI) registries. The data were analyzed to examine and compare demographic characteristics of those diagnosed with MPOX alone and those diagnosed with both HIV and MPOX. In addition, the HIV-MPOX co-morbid individuals were analyzed for HIV molecular cluster characteristics.

**Results:** The demographic characteristics of individuals diagnosed with MPOX, including MPOX-MPOX co-morbid individuals, mirror national trends: most were male, with a history of male-to-male sexual contact (MSM) and were likely to have received a recent STI diagnosis (DX). Individuals diagnosed with both HIV and MPOX were more likely to be included in HIV molecular clusters compared to three comparison groups: persons living with diagnosed HIV (PLWH) in ROS overall, MSM in ROS, and age-adjusted MSM (to match individuals with MPOX DX) in ROS. For the 3-year 0.5% clusters, which are used to define national priority clusters, the HIV-MPOX co-morbid individuals clustered 2.4 times more frequently than the age-risk-adjusted control group.

**Conclusion:** This study supports the use of HIV molecular clustering to identify individuals for priority public health interventions, including MPOX vaccination and testing.
injections were separated by 4 weeks. While there were protocol-specified visit windows for injections, some participants received injections outside of the visit schedule. Although CAB correlates of protection are unknown, we evaluated CAB concentrations in those with delayed injections during the blinded phase of HPTN 084 to assess the impact of less frequent dosing on CAB pharmacology.

Methods: Participants randomized to the CAB arm with at least one delayed injection were included in the analysis. Delayed injections were defined as type 1 (if the second injection occurred 8–14 weeks after the first injection; 4 weeks + 4–10) or type 2 (if a subsequent injection occurred 12–18 weeks after the preceding injection; 8 weeks + 4–10). CAB concentrations were measured at visits before and after an injection delay. Drug concentrations were evaluated relative to the 4x (0.664 mcg/mL) and 8x (1.33 mcg/mL) PA-IC90 (1.33 mcg/mL).

Results: One participant acquired HIV after a 16.1-week delay between injections and 9; CAB concentrations were < 4x PA-IC90 at the first HIV positive visit. We identified 194 participants who had at least one delayed injection (n=224 occurrences); 19ype 1 and 205 type 2 delays. For type 1 delays, 100% and 91% of occurrences yielded CAB concentrations ≥ 4x PA-IC90 and ≥ 8x PA-IC90, when an injection was given 8–10 weeks (4 weeks + 4–6) after the first injection; 75% were < 4x PA-IC90, when the delay was 12–14 weeks (4 weeks + 8–10). For type 2 delays, when the time between injections was 12–14 weeks (8 weeks + 4–6; m=109 occurrences), 98% and 87% of CAB concentrations were ≥ 4x PA-IC90 and ≥ 8x PA-IC90, respectively; this fell to 90% and 62% when injections were delayed 16–18 weeks (8 weeks + 8–10). Up to eight weeks post-administration of a type 2 injection delay, CAB concentrations were ≥ 4x PA-IC90 and ≥ 8x PA-IC90 in 100% and 94% of occurrences, respectively.

Conclusion: Pharmacologic analyses suggest there may be up to 6 weeks of forgiveness in persons assigned female at birth who received delayed CAB-LA injections. These data suggest that quarterly CAB-LA dosing in this population may be feasible and warrant further investigation.

THE LEVI SYNDROME: CHARACTERISTICS OF EARLY HIV INFECTION WITH CABOTEGRAVIR FOR PrEP

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Background: The LEVI syndrome was associated with diagnostic delays using standard local HIV testing algorithms, InStI resistance risk, and CAB-LA administration after infection. The prolonged viral suppression observed with CAB-LA PrEP suggests that there may be limited seeding of the HIV reservoir in early infection. Future studies are needed to evaluate the potential for HIV cure in this setting.

CABOTEGRAVIR FOR HIV PrEP IN US BLACK MEN AND TRANSGENDER WOMEN WHO HAVE SEX WITH MEN

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HPTN 083 Study Team
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Background: HIV infections are disproportionally higher among Black cisgender men and transgender women who have sex with men (MSM and TGW) compared with other racial/ethnic groups in the US. Long-acting injectable cabotegravir (CAB-LA) for HIV pre-exposure prophylaxis (PrEP) was shown to be safe and effective across populations globally. We evaluated HIV incidence and prevention efficacy of CAB-LA among US Black MSM and TGW.

Methods: HPTN 083 is an ongoing randomized controlled trial comparing CAB-LA to daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for HIV prevention in MSM and TGW. Eligible participants were randomized 1:1 to CAB-LA or TDF/FTC and followed for 153 weeks for incident HIV infection; TDF/FTC adherence was assessed among a subset of TDF/FTC-arm participants via plasma and dried blood spots (DBS).

Results: At US sites, 1698 MSM and TGW enrolled: 844 (49.7%) Black and 852 (50.2%) non-Black. The majority were MSM (92.6%), < 30 years old (61%), and non-Hispanic (82%). At study enrollment, Black and non-Black participants reported a similar number of sexual partners (mean: 3 vs 4), receptive anal sex acts in the past month (mean: 2 vs 3), and recreational drug use in the past 6 months (62.4% vs 72.6%); baseline prevalent syphilis was higher among Black participants (4.0% vs 1.9%). Among Black MSM and TGW, HIV incidence was 2.11% (95% Confidence Interval (CI): 1.18–3.48%) in the TDF/FTC arm and 0.58% (95% CI: 0.16-1.49%) in the CAB-LA arm [Hazard Ratio (HR): 0.28 (95% CI: 0.096-0.83)]. Among non-Black MSM and TGW, HIV incidence was 0.83% (95% CI: 0.00-0.44%) in the TDF/FTC arm compared with 0.00% (95% CI: 0.00-0.44%) in the CAB-LA arm [HR: 0.086 (95% CI: 0.004-2.060)]. In a subset of 151 participants, DBS-based metrics consistent with >4 doses/week of TDF/FTC were lower among Black (64.7%) than non-Black (81.2%) MSM and TGW. On-time CAB-LA injection was high (89.7%) and similar among Black and non-Black MSM and TGW.

Conclusion: In this US cohort of MSM and TGW, while HIV incidence was higher among Black participants in both study arms, the protective efficacy of CAB-LA vs. TDF/FTC was consistent. CAB-LA is a powerful HIV prevention tool to reduce HIV incidence among Black MSM and TGW, and implementation must focus on ensuring access and addressing disparities in HIV incidence among these populations.

CAB LA FOR HIV PREVENTION IN AFRICAN CISGENDER FEMALE ADOLESCENTS (HPTN 084-01)

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HPTN 08-01 Study Team
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Methods: Sexually active, HIV-uninfected females under 18 years of age, willing to use reliable long-acting contraception and weighing at least 35kg were eligible to participate. Parental/guardian consent was required for participation unless youth were considered emancipated minors. Step 1 involved a 5-week oral CAB 30mg QD lead-in, followed by 5 intramuscular gluteal injections of 3 mL (600 mg) of CAB-LA administered at weeks 5, 9, 17, 25 and 33 (Step 2). Participants then switched to daily oral TDF/FTC for 48 weeks of follow-up (Step 3) or opted to continue CAB-LA via open label extension (HPTN 084 OLE). We present safety data, injection tolerability, product acceptability, and adverse events (AEs) of special interest (Table 1).

Results: From November 2020-August 2021, 69 individuals were screened and 55 were enrolled (mean age=16, age range 12-17; 100% Black African). At baseline, most had a primary sexual partner in the past month (71%), 22% reported transactional sex, 31% had genitourinary chlamydia and 7% had gonorrhea. Fifty-two (95%) of participants completed Step 1 and 2; two participants discontinued during Step 3 for AEs unrelated to CAB-LA. One participant who started Step 2 stopped CAB-LA after 3 injections due to injection pregnancy. There were no product-related serious AEs or discontinuations from product due to AEs. No one acquired HIV on study. CAB-LA injections were well tolerated overall. Fourteen participants (26%) experienced 20 injection site reactions (ISR) – all Grade 1 or 2. For acceptability, 62% of participants reported via survey that they would consider using CAB-LA for HIV prevention in the future and 94% of participants went on to continue CAB-LA via HPTN 084 OLE.

Conclusion: CAB LA for HIV PrEP was found to be safe, tolerable and acceptable to sexually active adolescent females under the age of 18 in 3 African countries. Concurrent evaluation of CAB-LA for adolescents and adults has allowed for simultaneous licensure in several countries to date, expanding the HIV prevention options available to youth in circumstances of heightened risk.

Table 1. Adverse Events of Special Interest during Steps 1 and 2 (n=55 enrolled pts)

<table>
<thead>
<tr>
<th>Adverse Events of Special Interest during Steps 1 and 2 (n=55 enrolled pts)</th>
<th>Frequency of event reporting</th>
<th>Severity of event</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at injection site</td>
<td>Grade 1-2</td>
<td>All enrolled without intervention</td>
<td></td>
</tr>
<tr>
<td>Pain at implant site</td>
<td>Grade 1-2</td>
<td>All enrolled without intervention</td>
<td></td>
</tr>
<tr>
<td>Pain at implant site</td>
<td>Grade 0</td>
<td>All enrolled without intervention</td>
<td></td>
</tr>
<tr>
<td>Hematocrit increased</td>
<td>5</td>
<td>Grade 0-1</td>
<td></td>
</tr>
<tr>
<td>Anaplastic lymphoma</td>
<td>9</td>
<td>Grade 0-1</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>5</td>
<td>Grade 0-1</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>5</td>
<td>Grade 0-1</td>
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<td>Rash</td>
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<tr>
<td>Rash</td>
<td>5</td>
<td>Grade 0-1</td>
<td></td>
</tr>
</tbody>
</table>

CAB LA for HIV PrEP was found to be safe, tolerable and acceptable.
following administration (participants randomized to RT collection at 2h/48h or 24h/72h). EVG, TAF, and tenofovir (TFV) levels were measured in plasma, RF and RT homogenates; and TFV-DP in RT mononuclear cells. \textit{Ex vivo} HIV infectivity of RF explants was done pre- and post-dosing as a pharmacodynamic (PD) measure.

**Results:** Twenty-one participants without HIV (71% male) were enrolled and received study product (n=21 for 1 insert; n=19 for 2 inserts). TAF/ EVG inserts were safe; 9 participants reported 17 adverse events (AEs); one mild AE (anal erythema) was related. Median peak plasma EVG, TAF and TFV concentrations (concs) for 1/2 inserts were 7.2/3.29, 29.5/41.9, and 2.6/4.42 ng/mL, respectively. EVG and TFV were detected in RF in all samples at 2h; median values for 1/2 inserts at 24h were EVG 2234/2597 ng/mL and TFV 2909/8277 ng/mL. EVG and TFV were detected in nearly all RT homogenates at 2h post-dose; EVG conc declined and were BLO in 50%/30% for 1/2 inserts at 24 hours whereas TFV was measurable in the majority of samples for 72h. Median RT cell TFV-DP conc exceeded target levels for 72h. Compared to baseline, median cumulative log_{10} HIV p24 antigen was significantly reduced at all timepoints for both 1 and 2 inserts (p< 0.032 and p< 0.02, respectively), with median log_{10} declines with 2 inserts of -2.0, -1.8, -1.9, -1.7 at 2, 24, 48, and 72 h, respectively (Figure).

**Conclusion:** Rectal administration of 1 or 2 TAF/EVG inserts was safe and achieved high RF and RT levels of EVG and TFV with low systemic drug exposure. RT cell TFV-DP concentrations were higher than observed at steady-state with oral TDF dosing, with demonstrable inhibition of HIV infection for up to 72h. These results indicate that the TAF/EVG insert has great potential as an on-demand rectal microbicide for HIV prevention. Explant Challenge in Rectal Biopsy Supernatant: Median and inter-quartile range (boxplots) of the median log_{10} cumulative, weight-adjusted p24 levels from up to four rectal tissue explant supernatants per participant-timepoint. The gray horizontal bars represent the inter-quartile range (dark gray) and overall range (light gray) of the weight-adjusted LOD of cumulative p24 concentrations.

**166 ENSITRELVIR FOR MILD-TO-MODERATE COVID-19: PHASE 3 PART OF PHASE 2/3 STUDY**

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**Background:** Ensitrelvir is a SARS-CoV-2 3CL protease inhibitor approved in Japan under emergency regulatory approval system as an oral treatment for COVID-19. Here we report the key analysis results of 125 mg group of phase3 part (SCORPIO-SR).

**Methods:** This study was a multicenter, randomized, double-blind, placebo-controlled study. Regardless of SARS-CoV-2 vaccination status and presence of risk factors for severe disease, patients with mild-to-moderate COVID-19 within 120 hours from onset were randomized to oral administration of ensitrelvir 125 mg (375 mg loading dose on Day1), ensitrelvir 250 mg (750 mg loading dose on Day1), and placebo once daily, for 5 days. The primary endpoint was time to resolution of 5 symptoms of COVID-19 (stuffy or runny nose, sore throat, cough, feeling hot or feverish, and low energy or tiredness), and the key secondary endpoints include change from baseline on Day4 in the amount of SARS-CoV-2 viral RNA and time to first negative of viral titer. The primary population for the primary and key secondary endpoints was patients with <72 hours from onset to randomization.

**Results:** Median time to resolution of 5 symptoms was significantly shorter in 125 mg group (n=336, 167.9 hours) than placebo group (n=321, 192.2 hours) (p=0.0407). Mean change of viral RNA levels from baseline (log_{10} copies/mL) on Day4 was significantly greater in 125 mg group (2.48) than in placebo group (-1.01) (p< 0.0001). The time to first negative of viral titer was significantly shorter in 125 mg group (n=199, 36.2 hours) compared to placebo group (n=211, 65.3 hours) (p< 0.0001). Mean changes from baseline in viral titers (log_{10} (TCID_{50})/mL) were significantly greater in 125mg group on Day4 (-0.807, n=196) and Day4 (-1.108, n=197) than in the placebo group (-0.395, n=208 and -0.850, n=207, respectively) (p< 0.0001).

**165 ULTRA LONG-ACTING REFILLABLE ISLATRAVIR IMPLANT FULLY PROTECTS NHP AGAINST SHIV**

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**Background:** When taken as prescribed, antiretroviral (ARV) drugs are effective as pre-exposure prophylaxis (PrEP) against human immunodeficiency virus (HIV) transmission. However, consistent adherence to daily dosing regimens is challenged by issues such as pill fatigue, forgetfulness, stigma or lifestyle discordance. To this end, long-acting approaches with infrequent dosing intervals aim at improving therapeutic adherence and uptake. Among these, however, long-acting injectables suffer drawbacks of burst release, site-specific adverse reactions, and risky year-long sub-therapeutic tails. Moreover, injectables cannot be removed in the event of medical complications. Subdermal long-acting polymeric implants are also under investigations for HIV PrEP. However, such systems are commonly limited by decaying release profiles and require repeated surgical procedures for implantation and replacement upon drug exhaustion. To overcome these limitations, we developed a transcusaneous refillable ultra-long-acting islatravir delivery implant for HIV PrEP, based on a nonfluoridous silicon membrane technology.

**Methods:** We developed nanofabricated, biocompatible, silicon nanochannel membrane implants with channels in size of 280 nm. The implants achieved constant and sustained islatravir release via electrostatic confinement on molecules diffusing along the nanochannel path. The implant allowed for the transcusaneous loading and refilling of islatravir. A 20-month PK study was performed in vivo in rhesus macaques with implant subcutaneously inserted in the animal’s dorsum. Preventive efficacy was evaluated against rectal and vaginal infection using repeated SHIV challenges model in male and female rhesus macaques, respectively. Finally, implant toxicity and tolerability were assessed.

**Results:** Implants achieved constant islatravir plasma (median 3.14 nm) and islatravir-triphosphate (ISL-TP) (median 0.16 pmol/10^6 cells) levels for over 20 months uninterrupted, above the established PrEP protection benchmarks. In the PrEP efficacy studies with repeated low-dose SHIVSF162P3 challenges, the implants conferred 100% protection against rectal and vaginal infection. The implants were well tolerated with mild local tissue inflammation and no signs of systemic toxicity.

**Conclusion:** Our nanofluidic islatravir implant is a promising technology for HIV prevention, which may improve PrEP uptake, adherence and implementation.
Conclusion: Ensifertivir demonstrated a significant reduction in the time to resolution of 5 typical symptoms of COVID-19, robust antiviral effects and good tolerability.

167 EFFECT OF EARLY TREATMENT WITH PEGYLATED INTERFERON LAMDA FOR COVID-19

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The TOGETHER Investigators

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*Presented at CROI by a nonauthor colleague

Background: We assessed the efficacy of a single dose of peginterferon lambda in preventing clinical events among acutely symptomatic COVID-19 outpatients.

Methods: We conducted a placebo-controlled, randomized, adaptive platform trial among predominantly vaccinated SARS-CoV-2-positive adults in Brazil and Canada receiving either one subcutaneous injection of peginterferon lambda or placebo. The primary composite endpoint was medical admission to hospital, defined as either observation in a COVID-19 emergency setting for > 6 hours, or transfer to a tertiary hospital due to symptomatic COVID-19 within 28 days post-randomization.

Results: For this evaluation, 931 patients received peginterferon lambda and 1018 received placebo. 84% of the population were vaccinated and the trial occurred across multiple COVID-19 variants. In the primary analysis of patients, the primary outcome was reduced by 51% in the peginterferon lambda vs. placebo groups (relative risk 0.49 [25/916 vs 57/1003], 95% Bayesian credible interval 0.30-0.76, posterior probability >99.9%). This effect was maintained in subgroup analyses including COVID-19-related hospitalization alone (relative risk 0.57, 95% Bayesian credible intervals 0.33-0.95,) and COVID-19-related hospitalization or death (Hazard ratio 0.59, 95% Bayesian credible interval 0.35-0.97). The effects were consistent across dominant variants and vaccination status. Among individuals with a high viral level at baseline, peginterferon lambda resulted in lower viral loads by Day 7, compared to placebo. The incidence of adverse events was similar in the two groups.

Conclusion: Among predominantly vaccinated outpatients with COVID-19, single-dose of peginterferon lambda resulted in significantly decreased clinical events.

168 CHARACTERIZATION OF SINGLE VERSUS DUAL ACTIVE mAB AGAINST SARS-CoV-2

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Background: Amubarvimab and romlusevimab are anti-SARS-CoV-2 monoclonal antibodies (mAbs) that significantly reduced the risk of hospitalizations or death in the ACTIV-2/A5401 trial. SARS-CoV-2 variants (e.g., Delta, Epsilon, Lambda) harbor mutations against romlusevimab. We evaluated viral kinetics and resistance emergence in individuals treated with mono versus dual-active mAbs.

Methods: The study population included 789 non-hospitalized participants at high risk of progression to severe COVID-19 enrolled in the ACTIV-2/A5401 platform trial (NCT04518410) and received either placebo (n=400) or amubarvimab plus romlusevimab (n=389). Anterior nasal (AN) swabs were collected for SARS-CoV-2 RNA testing on days 0-14, and 28. Spike (S) gene next-generation sequencing were performed on samples collected at study entry and the last sample with viral load ≥2 log10 SARS-CoV-2 RNA copies per ml. We compared viral load kinetics and resistance emergence with single versus dual-active mAbs by categorizing participants as harboring variants sensitive to amubarvimab alone (Delta, Epsilon, Lambda) versus those sensitive to both mAbs (Alpha, Beta, Gamma, Others).

Results: Study participants receiving single and dual-active mAbs had similar demographics, baseline AN viral load, baseline symptom score and duration since symptom onset. The most common SARS-CoV-2 variant in the study population was Delta (26%) followed by Gamma (19%), Alpha (12%), and Epsilon (10%). In those with successful sequencing, 37% (N=111) were infected with a variant sensitive to amubarvimab alone and 63% (N=188) were infected with a variant sensitive to both mAbs. Compared to treatment with a single-active mAb, treatment with dual-active mAbs led to faster viral load decline at study day 3 (p=0.0001) and day 7 (p=0.003). Treatment-emergent resistance mutations were significantly more likely to be detected after amubarvimab plus romlusevimab treatment than placebo (2.6% vs 0%, P=0.0008). mAb resistance was also more frequently detected in the setting of single-active mAb treatment compared to dual-active mAb treatment (7.2% vs 1.1%, p=0.007). Participants with emerging mAb resistance had significantly higher pre-treatment SARS-CoV-2 nasal viral RNA levels.

Comparison: Compared to single-active mAb therapy, dual-active mAb therapy led to significantly faster viral load decline and lower risk of emerging mAb resistance. Combination mAb therapy should be prioritized for the next generation of mAb therapeutics.

169 SAFETY AND EFFECTIVENESS OF INHALED INTERFERON-β1A (SNG001) IN OUTPATIENTS WITH COVID-19

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ACTG A5401 Study Team
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Background: SARS-CoV-2 variants resistant to monoclonal antibodies, and drug-drug interactions and potential mutagenicity of direct acting antivirals, heightsen the need for additional therapeutics to prevent progression to severe COVID-19. Exogenous interferon beta is a promising therapeutic option against SARS-CoV-2 given its broad-spectrum antiviral activity and data suggesting impaired endogenous IFN production in individuals with severe disease.
Methods: The safety and efficacy of orally inhaled nebulized interferon-β1a (SNG001) was evaluated in a Phase II randomized controlled trial on the ACTIV-2/A5401 platform (NCT04518410). Adult outpatients with confirmed SARS-CoV-2 infection within 10 days of symptom onset were randomized to SNG001 once daily for 14 days or blinded pooled placebo. Primary outcomes included treatment-emergent Grade ≥3 adverse event (TEAE) through day 28; time to symptom improvement of 13 targeted COVID-19 symptoms collected by daily study diary through day 28; and SARS-CoV-2 RNA < lower limit of quantification (LLoQ) from nasopharyngeal (NP) swabs at days 3, 7, and 14. All-cause hospitalization or death through day 28 was a key secondary outcome.

Results: Of 221 participants enrolled at 25 US sites between February and August 2021, 220 (110 SNG001, 110 placebo) initiated study intervention, with a median age of 40 years, 55% female, and 20% SARS-CoV-2 vaccinated. There was no significant difference between SNG001 and placebo in Grade ≥3 TEAES (4% vs 8%, Fisher’s exact test p=0.25). Median time to symptom improvement was 13 days for SNG001 and 9 days for placebo ( Gehan-Williams test p=0.17). There was no difference in the proportion of participants with SARS-CoV-2 RNA < LLoQ at day 3, 7 or 14 (SNG001 vs placebo, Day 3: 28% vs. 39%; Day 7: 65% vs. 66%; Day 10: 91% vs. 91%; joint Wald test p=0.41). There were fewer hospitalizations with SNG001 (n=1) compared with placebo (n=7; 6%), but this difference was not statistically significant (Fisher’s exact test p=0.07, Figure). All hospitalizations were due to COVID-19 and occurred among unvaccinated participants without protocol-defined high-risk factors.

Conclusion: Inhaled nebulized SNG001 was safe and well tolerated but did not reduce SARS-CoV-2 RNA levels in the nasopharynx nor decrease time to improvement of COVID-19 symptoms in outpatients with mild-to-moderate COVID-19. The non-statistically significant decrease in hospitalizations among SNG001 participants warrants further investigation in a phase 3 clinical trial. Cumulative incidence of hospitalization or death comparing SNG001 vs. placebo CoV-2 nucleocapsid protein, with relative Ct values converted to absolute copy number via calibration to droplet digital PCR. A linear Tobit regression model was used to assess change over time while accounting for left censoring due to the viral load limit of detection. Results were adjusted for other treatment allocations within the factorial design, vaccination status, and baseline viral load. Repeated measures were accounted for using clustered standard errors within participants.

Results: Samples were available from n = 945, 871, and 775 participants on days 1, 5, and 10, respectively. The mean change from baseline to follow-up was -0.64 log10 copies/mL (95% CI -1.16 to -0.13) for metformin versus placebo, which equates to a 4.4-fold greater decrease. The mean change in SARS-CoV-2 from baseline to day 5 was -0.48 log10 copies/mL, and was -0.81 log10 copies/mL from baseline to day 10. The anti-viral effect increased with increased metformin dosing days 5-6. The antiviral effect was larger in those unvaccinated (mean -0.95 log copies/mL) than vaccinated (mean -0.39 log copies/mL). There was no change in viral load vs. placebo for ivermectin or fluvoxamine.

Conclusion: Metformin lowered SARS-CoV-2 viral load in this quadruple-blinded, randomized clinical trial. The temporal relationship to dose titration suggests a dose-dependent effect. The magnitude of antiviral effect was similar to niratreminovir at day 5, greater than niratreminovir at day 10. Metformin is safe, widely available, and has few contraindications.

171 SYMPTOM AND VIRAL REBOUND IN UNTREATED COVID-19 INFECTION Rinki Deo1, Manish C. Choudhary1, Carlee Moser1, Justin Ritz1, Eric S. Daar2,3, David A. Wohl4,5, Alexander L. Greninger6, Joseph J. Eron7, Judith S. Currier8, Michael D. Hughes9, Davey M. Smith7, Kara W. Chew6, Jonathan Z. Li9 ACTIV-2/A5401 Study Team 1 Brigham and Women’s Hospital, Cambridge, MA, USA, 2Harvard T.H. Chan School of Public Health, Boston, MA, USA, 3Harvard-University of California Los Angeles Medical Center, Torrance, CA, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 5University of Washington Medical Center, Seattle, WA, USA, 6University of California Los Angeles, Los Angeles, CA, USA, 7University of California San Diego, San Diego, CA, USA, 8Harvard Medical School, Cambridge, MA, USA

Background: Rebound of SARS-CoV-2 RNA and symptoms has been reported in people treated with niratreminovir/ritonavir. Since the natural course of viral and symptom trajectories during COVID-19 have not been well described, we evaluated the incidence of viral rebound and symptom relapse in untreated individuals with mild-to-moderate COVID-19.

Methods: This analysis included 563 participants randomized to placebo in the ACTIV-2/A5401 platform trial. Participants recorded the severity (scored 0-3) of each of 13 targeted symptoms daily from days 0-28, with symptom score being the summed score (0-39). Symptom rebound was defined as ≥4 point increase in symptom score between the maximum and the preceding minimum score. Anterior nasal (AN) swabs were collected for SARS-CoV-2 RNA testing on days 0-14 and 28. Viral rebound was defined as ≥2.0 log10 RNA copies/mL increase from the immediately preceding time point to a level ≥3.0 log10 RNA copies/mL, with high-level rebound defined as an increase of ≥2.5 log10 copies/mL to a level ≥5.0 log10 RNA copies/mL. To mirror the timing of a 5-day niratreminovir/ritonavir course, a supportive analysis was conducted where participants were only classified as rebounders if their rebounds occurred on or after day 5.

Results: Symptom rebound was identified in 26% of participants at a median [Q1, Q3] of 6 [4, 9] days after study entry and 11 [9, 14] days after initial symptom onset. Individuals with symptom rebound were more likely to be female, at high risk for progression to severe disease, have shorter time since symptom onset at study entry, and have higher symptom score and higher AN viral level day 0. Viral rebound was detected in 32%, with high-level rebound in 13% of participants. Participants with viral rebound were older, more likely to be female, at high risk for progression to severe disease, and had higher AN viral level at day 0. Most symptom and viral rebound were transient with 89% of symptom rebound and 95% of viral rebound events occurring for only a single day before improving. The combination of symptom and high-level viral rebound was observed in 3% of participants. In the supportive analysis of rebound occurring ≥5 days after study entry, 22% and 20% of participants met symptom and viral rebound criteria, respectively, but only 1.2% of participants met criteria for both symptom and high-level viral rebound.

Conclusion: Symptom or viral rebound in the absence of antiviral treatment is common, but the combination of symptom and viral rebound is rare.
COVID-19 HOSPITALIZATION RISK AFTER SARS-CoV-2 VACCINATION AND OUTPATIENT TREATMENT

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Background: Given effectiveness of SARS-CoV-2 vaccines and outpatient antiviral and monoclonal antibody therapy for reducing progression to severe COVID-19, we sought to estimate the impact of these interventions on risk of hospitalization following SARS-CoV-2 infection in a large US healthcare system.

Methods: All patients ≥18 of age in the UNC Health system, with first positive SARS-CoV-2 RT-PCR test or U07.1 ICD-10-CM diagnosis date during 07/01/2021-05/31/2022, were included. The outcome was first hospitalization with U07.1 ICD-10-CM primary diagnosis ≤14 days after SARS-CoV-2 diagnosis date. SARS-CoV-2 vaccinations were included if received ≥14 days prior to diagnosis. Outpatient therapies were included if administered after diagnosis date and before hospital admission. Age, gender, race, ethnicity, and comorbidities associated with COVID-19 (using ICD-10-CM, if documented ≥14 days prior to diagnosis date) were also evaluated. Risk ratios for hospitalization were estimated using generalized linear models, and predictors identified using extreme gradient boosting using feature influence with Shapley additive explanations algorithm.

Results: The study population included 54,886 patients, 41% men and 27% ≥60 years of age. One-third of SARS-CoV-2 diagnoses occurred July-December 2021 and 67% December-May 2022 (predominantly Delta and Omicron variants, respectively). Overall 7.0% of patients were hospitalized for COVID-19, with median hospitalization stay of 5 days (IQR: 3-9). 32% and 12% of patients received ≥1 SARS-CoV-2 vaccine dose and outpatient therapy, respectively. Unadjusted and age-adjusted hospitalization risk decreased with vaccination and outpatient therapy (TABLE). Comparing patients who received 3 vaccine doses versus none we observed a 66% relative reduction in risk, with stronger association for more recent vaccination. For patients who received nirmatrelvir/ritonavir versus no therapy we observed a 99% relative reduction in risk.

Conclusion: The impact of recent SARS-CoV-2 vaccination and outpatient antiviral and monoclonal antibody therapy on reducing COVID-19 hospitalization risk was striking in this large healthcare system covering Delta and Omicron variant timesframes. SARS-CoV-2 vaccinations and outpatient therapeutics are critical for preventing severe COVID-19.

Unadjusted and age-adjusted risk ratios for hospitalization among patients with SARS-CoV-2

<table>
<thead>
<tr>
<th>Risk Ratio (95% CI)</th>
<th>N</th>
<th>Unadjusted</th>
<th>Age adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2 vaccine doses vs. no vaccine</td>
<td>1</td>
<td>2.129</td>
<td>0.38 (0.30-0.48)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.099</td>
<td>0.40 (0.36-0.45)</td>
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<tr>
<td></td>
<td>3</td>
<td>5.010</td>
<td>0.24 (0.20-0.28)</td>
</tr>
<tr>
<td>Time from last SARS-CoV-2 vaccine dose vs. ≥90 days</td>
<td>91-180 days</td>
<td>4.994</td>
<td>1.08 (0.85-1.38)</td>
</tr>
<tr>
<td></td>
<td>181-270 days</td>
<td>4.635</td>
<td>1.13 (0.88-1.44)</td>
</tr>
<tr>
<td></td>
<td>&gt;270 days</td>
<td>3.664</td>
<td>1.42 (1.13-1.81)</td>
</tr>
<tr>
<td>Outpatient SARS-CoV-2 therapy vs. no therapy</td>
<td>Cardiokininamine &amp; nirmatrelvir</td>
<td>3.589</td>
<td>0.27 (0.21-0.34)</td>
</tr>
<tr>
<td></td>
<td>Nirmatrelvir &amp; niveronn</td>
<td>1.230</td>
<td>0.01 (0.001-0.04)</td>
</tr>
</tbody>
</table>

MPOX IN PEOPLE LIVING WITH HIV AND CD4 COUNTS < 350 CELLS/MM3: A GLOBAL CASE SERIES

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Background: People living with HIV (PLWH) represent 38-50% of the 2022 global MPox outbreak. Most PLWH included in case series had CD4 counts >500 cells/mm³ and similar outcomes to persons without HIV. Existing data suggest poorer outcomes in immunosuppressed PLWH. 60 deaths with MPX outside of Africa were reported in 2022—only 9 have been described and published. We describe clinical characteristics and deaths in PLWH with low CD4 cell counts (< 350 cells/mm³) and MPX.

Methods: International collaborators from 18 countries contributed data from PLWH with CD4 counts < 350 cells/mm³ & confirmed MPX between May 11th and December 24th, 2022. We describe in detail the clinical course, complications & causes of death with respect to both CD4 & viral load (VL) strata. Analyses were descriptive (continuous variables described as mean and standard deviation (SD); categorical variables as counts & percentages).

Results: We report on 258 persons (mean age 36 years): 251 cisgender men, 2 cisgender women, 5 transgender women. At (MPX) diagnosis, 234 were known PLWH (205/234 on ART); 24/258 were undiagnosed, 26/258 had a concurrent opportunistic illness. Mean CD4 count was 210 cells/mm³ (SD 96); mean VL was 1.6 log c/mL (SD 2). A total of 39 (15%), 70 (27%), 84 (33%), 53 (21%) persons had CD4 counts of < 100, 100-200, 200-300, & >300 cells/mm³, respectively. 66/258 persons were hospitalised with MPox, of whom 1 survived an ITU admission & 14 died. Among those who died, the mean CD4 count was 63 cells/mm³, mean VL was 4.5 log c/mL. 13/14 had severe coalescing/necrotising skin lesions, secondary bacterial infections and rectal complications, 10/14 had respiratory symptoms and respiratory failure, 5/14 had neurological involvement (all described as confusion not encephalitis). More deaths occurred in persons with lower CD4 counts and higher viral loads (CD4 < 100, 18% vs. CD4 >300, 0%); & (VL ≥4 log 16% vs. VL < 50 c/mL 0.7%) respectively (Figure). More complications occurred in 39 persons with CD4 < 100 compared to 53 persons with CD4 >300 cells/mm³: respiratory (59% vs 25%), rectal (59% vs 25%), skin (51% vs 8%), bacterial (38% vs 9%), gastro intestinal (26% vs 6%), CNS (10% vs 2%). Immune reconstitution inflammatory syndrome was suspected in 15/46 persons started or re-started on ART with MPX.

Conclusion: In our case series in PLWH with MPX, severe systemic complications and deaths occurred most commonly in persons with CD4 < 100 cells/mm³ and viremia.

FIGURE. OUTCOME BY CD4 COUNT (A) AND HIV VIRAL LOAD (B)

CD8+ T CELLS INDUCE HIV LATENCY IN CD4+ T CELLS THROUGH THE DOWNMODULATION OF NF-κB

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Background: The key barrier to achieving either a cure for HIV infection or a remission in the absence of antiretroviral therapy (ART) is the presence of cells harboring integrated, replication competent virus (i.e. the reservoir) that persists despite long-term, fully suppressive ART. Although previous studies have demonstrated that CD8+ T cells negatively regulate HIV expression and may influence HIV latency, the exact mechanism is unknown. Here we identify the HIV transcriptional regulator NF-κB as a key target modulated by non-cytolytic CD8+ T cells.

Methods: We adapted our previously developed primary cell-based in vitro model of HIV latency to study the molecular mechanism exerted by CD8+ T cells for the suppression of HIV expression in memory CD4+ T cells (mCD4+). Resting or TCR-activated mCD4+ T cells were HIV infected in vitro and then co-cultured with autologous activated total CD8+ T cells in the presence of ART. mCD4+ T cell monocultures were included as controls. After 24-hour co-culture, mCD4+ and CD8+ T cells were sorted by FACS and mCD4+ were returned to in vitro culture for an additional 72 hours. HIV expression was monitored by
in intracellular Gag and infection frequency was quantified by integrated HIV DNA quantitative PCR. Modulation of NF-κB was monitored by RT-PCR of NF-κB targets at 24, 48 and 72 hours post-culture.

Results: Following co-culture with autologous activated CD8+ T cells, both resting and activated mCD4+ T cells showed a significant reduction in Gag expression compared to the monocytes. The downmodulation of HIV expression was comparable among the mCD4+ T central, transitional and effector subsets. To determine if the suppression of HIV expression exerted by CD8+ T cells is NF-κB dependent, we measured the expression of the NF-κB targets IL-6, IFN-γ, and the canonical p65. The NF-κB activity was significantly reduced in both resting and activated mCD4+ T cells following exposure to CD8+ T cells and its downmodulation was maintained after CD8+ T cells removal from coculture.

Conclusion: Our results show that CD8+ T cells play a pivotal role in the establishment of HIV latency by downmodulating HIV expression. CD8+ T cells modulate the NF-κB transcriptional activity in mCD4+ T cells independently from HIV infection, thus suggesting that this pro-latency activity might be due to the NF-κB suppression. Understanding the mechanism responsible for the NF-κB modulation in mCD4+ T cells may represent a tool to reverse the HIV latency and develop a new cure strategy.

175 BLOCKING CD4+ T CELL MIGRATION DELAYS VIRAL SPREAD FOLLOWING VAGINAL SIV CHALLENGE
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Background: Nearly 2 million new HIV infections occur each year from sexual transmission, yet little is known about the anatomic steps that lead to systemic dissemination following vaginal exposure. We previously showed that following SIV vaginal challenge, CD4+ T-cell density increases in the female genital tract (FGT), and that local viral replication in FGT necessarily precedes dissemination. We hypothesize that cells harboring infectious virus are the predominant mechanism of viral dissemination.

Methods: To address this, we vaginally challenged eight rhesus macaques (RM) with the sequence tagged synthetic swarm SIVmac239X. Six animals were pre-treated with fingolimod (FTY720) to block the egress of leucocytes out of tissues. Animals were euthanized two weeks post-challenge. Plasma viral load was monitored frequently, and sequence analysis was used to assess the number of transmitted/founder lineages. T-cell populations were assessed using flow cytometry and immunohistochemistry.

Results: We observed a significant delay in the time to measurable viremia in animals receiving fingolimod (up to 5 days) as well as a drastic reduction in viral load magnitude at the time of necropsy (up to 5 logs). We observed a striking reduction in the number of CD4+ T cells (and all leucocytes) in the blood following fingolimod treatment with an abundance of CD4+ T cells retained in lymph nodes. However, when quantifying the density of CD4+ T cells within the FGT, there was no significant difference in treated vs non-treated animals. In contrast, CD8+ T cells were more abundant in the vagina tissue of non-treated animals. Sequencing of plasma virus revealed that animals treated with fingolimod were systemically infected with only one to two different variants, whereas the non-treated animals were infected with an average of five distinct variants.

Conclusion: Restricting trafficking of CD4+ T cells impeded the transition of local infection to systemic infection as demonstrated by significantly decreased and delayed plasma viremia. These differences were not due to differences in the availability of target cells at the challenge site nor were they likely due to any potential adaptive immune effects prior to dissemination. These observations highlight the importance of the mobility of productively infected cells and that systemic spread of virus via cell-free virion dissemination is slower and less efficient.

176 DEPLETION OF SIV-SPECIFIC CD8+ T CELLS DOES NOT ALTER VIRAL LOAD DURING SIV INFECTION
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Background: The emergence of SIV-specific CD8+ T cells coincides with declining viremia in monkeys acutely infected with SIV. Antiviral CD8+ T cells may also contribute to immune control in chronically infected animals treated with antiretrovirals (ARVs). We explored these relationships using toxin-conjugated MHC class I tetramers to deplete Gag CM9-specific CD8+ T cells in Mamu-A01+ rhesus macaques infected with a pathogenic strain of SIV.

Methods: Mamu-A01+ rhesus macaques were infected with SIVmac239. Gag CM9 (CTPDPINQM) tetramers conjugated to saporin were administrated to animals with progressive infection and animals with spontaneous control of viremia to <10,000 copies/mL. Lymphocyte populations in blood, bronchoalveolar lavage fluid, lymph nodes, colon, and jejunum were enumerated via flow cytometry. Plasma viremia was measured via quantitative RT-PCR.

Results: Immunotoxin-conjugated CM9/Mamu-A01 tetramers induced a transient and significant depletion of CM9-specific CD8+ T cells in blood with lesser effects in tissues. This manipulation resulted in viral recrudescence in one animal with spontaneous control of viremia but did not alter the kinetics or magnitude of viremia in other animals during acute or chronic infection, irrespective of treatment with ARVs.

Conclusion: Immunotoxin-conjugated MHC class I tetramers effectively induced a transient depletion of circulating SIV-specific CD8+ T cells. Our results suggest that CM9-specific CD8+ T cells are important for elite control of viral replication but play a minimal role in the transition from acute to set-point viremia in animals with progressive infection. No evidence was found to support the notion that SIV-specific CD8+ T cells contributed to the reduced viremia observed in animals treated with ARVs.

177 PROTECTION AGAINST HTLV-1 CHALLENGE BY VACCINATION INDUCING ANTIBODIES IN MACAQUES
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1National Institute of Infectious Diseases, Shinyaku, Japan; 2National Institute of Infectious Diseases, Tokyo, Japan; 3Kumamoto University, Kumamoto, Japan; 4National Institute of Infectious Diseases, Musashimurayama, Japan

Background: Human T-cell leukemia virus type 1 (HTLV-1) causes severe diseases such as adult T-cell leukemia (ATL) and HTLV-1–associated myelopathy in some individuals after long-term asymptomatic phase of latent infection. Control of horizontal HTLV-1 transmission is a globally important issue, and there is a demand for the development of an effective HTLV-1 vaccine. In HTLV-1 transmission, latently HTLV-1-infected cells are transmitted into the donor, resulting in cell-to-cell virus transmission. It is a great challenge to determine whether vaccine-induced anti-Env antibodies can protect the cell-to-cell HTLV-1 transmission. In the present study, we examined the protective efficacy of a vaccine inducing anti-Env antibodies against HTLV-1 challenge.

Methods: We have recently developed a Sendai virus (SeV) vector expressing a chimeric antigen consisting of the HTLV-1 gp63 ectodomain and the SeV transmembrane–cytoplasmic domain (HTLV-Env), which can be incorporated into the SeV virion. A vaccine using the SeV-HTLV-Env and a non-infectious SeV particle carrying HtvlEnvF (NVP-HtvlEnvF) has been shown to induce anti-HTLV-1 neutralizing antibodies in mice (Vaccine, 40, 2420-2431, 2022). In this study, we examined the protective efficacy of the vaccine using SeV-HTLV-EnvF against an intravenous challenge with 108 HTLV-1–producing cells (an ATL cell line provided by Dr. Yuetusa Tanaka) in cynomolgus macaques.

Results: In the first experiment, three of the five cynomolgus macaques vaccinated with SeV-HTLV-EnvF and NVP-HTLV-EnvF induced substantial anti-HTLV-1 neutralizing antibodies (NAb) and were protected from the challenge. In the second experiment, all the five cynomolgus macaques vaccinated subcutaneously with SeV-HTLV-EnvF induced substantial anti-HTLV-1 NAb, and were protected from the challenge. In contrast, all the unvaccinated cynomolgus macaques (n = 10) were infected with detectable proviruses.

Conclusion: These results indicate that neutralizing antibody induction by vaccination can result in protection from HTLV-1 transmission, implying...
178 SHIFTS IN SYSTEMIC INFLAMMATION AFTER FECAL MICROBIOTA TRANSPLANT IN PEOPLE WITH HIV
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1Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain, 2Hospital Ramón y Cajal, Madrid, Spain, 3UCAIB, Valencia, Spain

Background: Whether interventions on the microbiota can help reduce chronic inflammation during treated HIV infection remains unclear. In a fecal microbiota transplant (FMT) trial in PWH, we found that Lachnospiraceae and Ruminococcaceae families—with known anti-inflammatory properties—were the taxa more robustly engrafted across time points. Here, we aimed to better characterize the FMT effects on systemic inflammation.

Methods: We randomized 30 PWH on ART to either weekly fecal microbiota capsules or a placebo for eight weeks. Stool donors were selected based on anti-inflammatory microbiota profiles (high Faecalibacterium and butyrate abundance). We measured the plasma expression of 368 inflammatory proteins at weeks 0, 1, 8, and 24 using the Proximity Extension Assay by Olink. We fitted mixed models to compare the protein trajectories and selected the most significant to explore their correlations with Lachnospiraceae and Ruminococcaceae families. We performed a functional analysis using Gene Set Enrichment Analysis.

Results: FMT resulted in a significant decrease of 24 proteins related to cytokines and leukocyte activation compared to placebo. CCL22, CD22, FGF5, and JUN showed the most consistent pattern of decline after each FMT; CCL22, IL20, and JUN were those with the most apparent effect at week 24 (17 weeks after the last FMT); and AOC1 and IL13 showed the most significant differences between groups (p=0.0045 and p=0.0003)(Figure 1A). From these 24 proteins, increases in Lachnospiraceae and Ruminococcaceae families correlated with increased CCL22 (p=0.0094) and decreased IL20 expression (p = 0.0255) (Figure 1B-C).

Conclusion: Repeated FMT downregulated the expression of inflammatory proteins related to cytokines and leukocyte activation. The effects of FMT on Lachnospiraceae and Ruminococcaceae abundance correlated with CCL22 and IL20 kinetics, with known roles on Th17 cell preservation in HIV and the immune response against bacterial infections. Our results support investigating interventions on the microbiome to modulate systemic inflammation in PWH on effective ART.

Figure 1. A. Observed changes in normalized CCL22 and IL20 expression in each group. B. Associations between weekly changes in Lachnospiraceae and Ruminococcaceae (scaled abundance) and CCL22 and IL20 (normalized expression) weekly changes estimated from linear mixed models with interaction terms with time. Dashed lines represent individual correlations between bacteria and protein changes and black lines the coefficients obtained from mixed models.

179 AUTOLOGOUS NEUTRALIZING ANTIBODY RESPONSES IN bNAB-TREATED RHESUS MACAQUES
Elise G. Viox1, Ryan Krause2, Emily Lindemuth2, Jin Wang2, Sadia Samer1, Kevin Nguyen1, Steffen Docken1, Suwadi Malik2, Brandon F. Keele1, Michael R. Betts1, Miles Davenport2, Mirko Paiardini1, Katharine Bar1
1Emory University, Atlanta, GA, USA, 2University of Pennsylvania, Philadelphia, PA, USA, 3Kirby Institute, Sydney, Australia, 4Northwestern University, Chicago, IL, USA, 5Frederick National Laboratory for Cancer Research, Frederick, MD, USA

Background: Broadly neutralizing antibodies (bAbs) are important tools for HIV-1 therapy and cure strategies, yet the role of autologous neutralizing antibodies (anAbs) during bNab therapy is unclear. We studied the kinetics of anAb responses during VRC07523.LS therapy at analytical treatment interruption (ATI) in a robust SHIV-infected Rhesus Macaque (RM) model of HIV-1.

Methods: 18 RMs infected IV with 1x106 infectious units of SHIV.D191859 (SHIV.D) imitated antiretroviral therapy (ART) at 120 days post-infection (dpi). After 6 months of ART, all 18 RM underwent ATI, during which 9 were administered 30 mg/kg VRC07523.LS. Plasma virus env sequences were derived by single genome sequencing. SHIV.D and rebound Env were tested for neutralization sensitivity.

Results: 18 RM experienced peak and pre-ART viral loads of 4.9x105 and 3.9x107 copies/mL, respectively. Virus was suppressed within 3 weeks of ART and rebounded in all RM following ATI, with a significant delay in VRC07523.LS-resistant rebound virus escape in all RM. Plasma virus env sequences pre-ART and at rebound (median 31 sequences per RM) revealed 1-5 virus lineages at rebound. Rebound Env was universally resistant to 120dpi plasma IgG (p=0.0025, vs. SHIV.D anAbs).

Conclusion: SHIV.D-infected RMs demonstrate viral kinetics, anAb responses, and effects of CD4bs bNab monotherapy that closely mirror human trials. Rebound virus escaped baseline anAb responses, suggesting ongoing humoral immune pressure at ATI. Virus rebound preferentially boosted anAbs against inoculum vs. rebound virus, indicating antibody imprinting, or original antigenic sin. AnAb potencies approaching those of VRC07523.LS suggest a potential role for anAbs to restrict archived virus and complement bNAb-based interventions.
ANALYSIS OF EPITOPE-SPECIFIC T CELLS IN SARS-COV-2 INFECTION AND VACCINATION
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Background: T cells play a critical role in the adaptive immune response to SARS-CoV-2 in both infection and vaccination. Identifying T cell epitopes and understanding how T cells recognize these epitopes can help inform future vaccine design and provide insight into T cell recognition of newly emerging variants. Here, we identified SARS-CoV-2-specific T cell epitopes, analyzed epitope-specific T cell repertoires, and characterized the potency and cross-reactivity of T cell clones across different common human coronaviruses (HCoVs).

Methods: SARS-CoV-2-specific T cell epitopes were determined by IFNγ ELISpot using PBMC from convalescent individuals with mild/moderate disease (n=25 for Spike (S)), Nucleocapsid (N) and Membrane (M)), and in vaccinated individuals (n=27 for S). Epitope-specific T cells were isolated based on activation markers following a 6-hour peptide stimulation, and scRNAseq was performed for TCR repertoire analysis. T cell lines were generated by expressing recombinant TCRs in Jurkat cells and activation was measured by CD69 upregulation.

Results: We identified multiple immunodominant T cell epitopes across S, N and M proteins in convalescent individuals. In vaccinated individuals, we detected many of the same dominant S-specific epitopes at similar frequencies as compared to convalescent individuals. T cell response to peptide S205 (amino acids 817-831) were observed in 56% and 59% of individuals following infection and vaccination, respectively, while 20% and 19% of individuals responded to S302 (a.a. 1205-1219) following infection and vaccination, respectively. For S205, a CD4+ T cell response, we confirmed 8 unique TCRs and determined the minimal epitope to be a 9mer (IEDLLFNKV). While TCR genes TRAV8-6*01 and TRBV30*01 were commonly utilized across the TCRs, we did identify TCRs with unique immunogenetic properties with different potencies of cross-reactivity to other HCoVs. For S302, a CD8+ T cell response, we identified two unique TCRs with different immunogenetic properties that recognized the same 9mer (YIKWPWYYW) and cross-reacted with different HCoV peptides (Figure 1).

Conclusion: These data identify immunodominant T cell epitopes following SARS-CoV-2 infection and vaccination and provide a detailed analysis of epitope-specific TCR repertoires. The prospect of developing a vaccine that broadly protects against multiple human coronaviruses is bolstered by the identification of conserved immunodominant SARS-CoV-2 T cell epitopes that cross react with multiple other HCoVs.
182 SINGLE INFUSION OF STEM LIKE CCR5-MODIFIED CD4 T CELLS PROVIDE LONG-TERM HIV CONTROL
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Background: Antiretroviral therapy (ART) fails to fully restore immune function and is not curative, therefore more effective therapies are required for people with HIV (PWH). A cure for HIV has previously been observed in several individuals in which bone marrow transfer of CCR5-modified stem cells for leukemia treatment was also found to eliminate HIV. While these results are encouraging, a less invasive and more broadly applicable curative strategy is warranted. Transfer of autologous CCR5-modified CD4 T cells has been found to be safe and well-tolerated in PWH. However, whether this treatment reprograms the immune response to provide long-term viral control is unknown.

Methods: We performed two clinical studies, SB-728-902 (n = 9) and SB-728-1101 (n = 9), in which participants were single infusions of autologous CCR5-modified T cells and ART was either maintained or interrupted (ATI), respectively. At 3-6 timepoints over 6 years, we collected blood samples and comprehensively profiled the immune response using multi-parameter flow cytometry and single-cell RNA sequencing.

Results: A single infusion of autologous CCR5-modified CD4 T cells led to a significant increase in absolute CD4 T cell count (+182 cells/μL, p = 0.002), reduced integrated HIV DNA (P = 0.004) in patients on ART (SB-728-902) and control of plasma viremia (for 1 to 6 years) upon ATI in 5 participants (SB-728-1101). These outcomes were associated with high frequencies of CCR5-modified CD45RAintCD45ROint CD4 T cells that had a quiescent metabolic profile (high oxidative phosphorylation) and were enriched in pathways/markers that regulate stemness (i.e., TCF1 protein, WNT/b-catenin signaling and TGF-b cascades). Single-cell trajectory analyses showed that this population gave rise to an effector CD4 T cell population which expressed high interferon inducible anti-viral genes. Higher frequencies of this subset were associated with both heightened effector CD8 T cell responses and reduced viral load.

Conclusion: Our results indicate that a single infusion of autologous CCR5-modified CD4 T products that are enriched in the novel CD45RAintCD45ROint stem-like phenotype are protected against HIV "re-infection" because they express mutated CCR5, which favors this anti-viral signature. This protection from infection promotes long-lasting effector CD48/8 T cell responses that can control viremia and facilitate a functional cure against HIV.

183 SEX MODIFIES THE AGE OF ARIEOMIA WITH STROKE RISK IN HIV
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1CFAR Network of Integrated Clinical Systems (CNICS) Network, 2University of California San Francisco, San Francisco, CA, USA, 3University of Washington, Seattle, WA, USA, 4VA Puget Sound Health Care System, Seattle, WA, USA, 5University of California San Diego, San Diego, CA, USA, 6University of Alabama at Birmingham, Birmingham, AL, USA, 7University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 8University of Manitoba, Winnipeg, MB, Canada

Background: The increased risk of stroke conferred by HIV may be greater for women than for men. Little is known about potential mechanisms driving the differential stroke risk by sex in people with HIV (PWH). We examined whether sex modifies the effect of traditional and HIV-related risk factors associated with stroke in PWH.

Methods: We analyzed the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort of PWH receiving HIV care. Strokes occurring in PWH from 5 sites across the U.S. were centrally adjudicated by neurologists. Follow-up in care was from –2005-2020 (end dates varied by site). The observation period began at the date of stroke surveillance by site or the initial CNICS visit date plus 6 months and ended at the earliest of: stroke, last visit plus 9 months, death, or administrative censoring date. Data from the CNICS central data repository included demographics, laboratory values, medication prescriptions, and diagnoses. Substance use, depression, and physical activity were from the CNICS clinical assessment of patient-reported outcomes collected as part of care visits. Cox survival models were used to assess the hazard ratio of stroke for predictors of interest, female sex, and the interaction between predictors of interest and female sex adjusted for age, race/ethnicity, and site.

Results: Among 13,584 PWH (mean age 44 years, 19% women, 40% Black, 81% with viral load < 400 copies/mL), there were 147 incident strokes during follow-up (mean follow-up 5.8 years for women, 5.5 years for men). In the overall cohort, age (HR 1.63 per 10 years, 95% CI 1.34-1.98, p< 0.001) but not sex (HR 1.19 for women compared to men, 95% CI 0.77-1.82, p = 0.43) was a risk factor for stroke. However, a statistically significant age-by-sex interaction was observed (p = 0.005). At younger ages, the risk of stroke was higher for women compared with men (HR 2.09 for women versus men at age 40, 95% CI 1.27-3.44, p = 0.004), whereas with older age, this difference was no longer present. Conversely, the risk of stroke associated with having a detectable viral load or using methamphetamines was greater for women than for men (Table).

Conclusion: Stroke risk was higher for some women with HIV compared with men, although this effect declined with older age. The effect of other risk factors on stroke, including viremia, differed between women and men with HIV. Investigation into the mechanisms underlying these differences and how this may translate into sex-specific stroke treatment and prevention is warranted.

Differences in the associations of risk factors with sex

<table>
<thead>
<tr>
<th>Table: Differences in the associations of risk factors with stroke by sex</th>
</tr>
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<tbody>
<tr>
<td>Predictors</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
</tr>
<tr>
<td>Female sex</td>
</tr>
<tr>
<td>Age on sex interaction</td>
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</tbody>
</table>

*All models are adjusted for age, race/ethnicity, and site.

1The HR for the predictor among men (e.g., among women, there is a 2.09-fold higher hazard of stroke for every 10 years of age, among men, methamphetamine use is associated with a non-significantly 1.19-fold higher hazard of stroke).

2The contrast reflects the sex difference in risk for stroke, with the HR for the interaction term reflects the difference between the HR for stroke in women versus men (p < 0.001) after adjusting for age, sex, race/ethnicity, and site.

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1INRA, 2Lyon, 3Vanderbilt University, Nashville, TN, USA, 4University of California San Francisco, San Francisco, CA, USA, 5Centre de Recherche du CHUM, Montreal, QC, Canada

Background: Persistent systemic and CNS immune activation, as well as signs of neuronal injury, are commonly found in people living with HIV (PLWH) despite suppressive antiretroviral therapy. We have previously shown that HIV-negative persons on PrEP had higher levels of biomarkers for immune activation, blood-brain barrier (BBB) impairment and neuronal injury, compared with volunteers without PrEP. The aim of this study was to explore markers of immune activation and neuronal injury in ART-treated PLWH compared to controls with similar lifestyle-related factors.

Methods: Cerebrospinal fluid (CSF) and blood were collected from 50 men who have sex with men (MSM) treated with ART >12 months (median age 37.8, 24-68), 50 HIV-negative MSM on PrEP (35.5, 22-68), and 35 HIV-negative healthy volunteers (44.3, 22-68). Biomarkers of immune activation (CD38, CD107a, CD105, HLA-DR), BBB microglobulin and neopterin), BBB integrity (CSF/plasma albumin ratio) and neuronal injury (neurofilament light protein, NfL) were analyzed.

Results: All 50 HIV-infected MSM on ART had HIV-RNA < 50 c/mL in plasma and CSF. Their CD4-cell counts (median 670, IQR 482-895 cells/μL) and CD4/CD8 ratio (0.94, 0.74-1.16) were significantly lower (p < 0.001) than the PrEP (850, 2023).
620-1100 and 1.33, 1.0-1.7) and volunteer (810, 660-1100, 1.52, 1.4-1.9) groups while no significant difference was seen in CD8 cell counts. No significant differences in serum and CSF β2-microglobulin or neopterin were found between the PLWH and PreP groups. However, both groups had significantly higher serum levels of β2-microglobulin (1.9, 1.7-2.3 and 1.9, 1.7-2.2 mg/L, p<0.001) and neopterin (8.6, 6.0-12.6 and 8.65, 6.3-11.9 nmol/L, p<0.01) compared to HIV-negative volunteers not on PreP (1.5, 1.3-1.6 and 6.9, 5.7-7.3). Age adjusted CSF NFL was also higher in the PLWH (347, 294-456 ng/L, p<0.01) and MSM PreP (371, 290-544, p<0.001) groups compared to voluntary controls (255, 208-338), while no significant differences were found in CSF β2-microglobulin, CSF neopterin or albumin ratio.

Conclusion: We found increased markers of immune activation and neuronal injury in both virologically suppressed PLWH and HIV-negative persons on PreP compared to HIV-negative volunteers. These results indicate that factors unrelated to HIV-infection may contribute to the persistent immune activation and CNS injury commonly seen in PLWH on ART and highlights the importance of using appropriate control groups with comparable lifestyle-related factors.

Figure

185 CEREBROSPINAL FLUID HIV RNA AND VIRAL NUCLEIC ACID DETECTION IN PERSONS WITH HIV

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1Imperial College London, London, United Kingdom, 2Imperial College Healthcare NHS Trust, London, United Kingdom

Background: Data on the prevalence of cerebrospinal fluid (CSF) HIV RNA escape and other viral nucleic acid detection in the modern anti-retroviral therapy (ART) era are sparse. Our aim was to determine the recent incidence of CSF HIV RNA escape and other viral nucleic acid detection in persons with HIV undergoing a lumbar puncture examination for clinical indications over a 5-year period and assess the associated clinical factors.

Methods: Persons with HIV with CSF virology results at a large London centre were identified from pathology records between 2017-2022 and clinical data recorded. CSF HIV RNA escape was defined as CSF HIV RNA concentrations greater than concurrent plasma HIV RNA. CSF viral screen included herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), varicella zoster virus (VZV), Epstein Barr virus (EBV), cytomegalovirus (CMV), human herpes virus 6 (HHV-6), JC virus, adenovirus, enterovirus and parechovirus. For viruses that were detected in >5% of the study population, absolute counts were calculated. The median viral load (medium [IQR]) was calculated for HSV-1, HSV-2, EBV, CMV, HHV-6, JC virus, adenovirus, enterovirus, parechovirus, and hepatitis C virus.

Results: Of 114 individuals, clinical indication for lumbar puncture examination included new onset neurological symptoms (n=38), investigation for neurosyphilis (n=24), new onset psychiatric symptoms (n=6) and new findings on brain MRI (n=1). CSF HIV RNA escape was observed in 19 (17%, table) and in the absence of clinical manifestations may be a consequence of CSF pleocytosis and trafficking of viral nucleic acid into the CSF compartment.

Table 1: Baseline demographic, clinical and laboratory characteristics of the total cohort and those with and without CSF HIV RNA escape

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>Total cohort</th>
<th>n=114</th>
<th>CSF HIV RNA escape</th>
<th>n=19</th>
<th>CSF HIV RNA non-escape</th>
<th>n=95</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>median (IQR)</td>
<td>40.6 (23-54)</td>
<td>39.5 (23-54)</td>
<td>41.9 (23-54)</td>
<td>0.346</td>
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<tr>
<td>Gender, male</td>
<td>%</td>
<td>83 (71)</td>
<td>73 (71)</td>
<td>85 (76)</td>
<td>0.036</td>
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<td>Ethnicity, caucasian</td>
<td>%</td>
<td>49 (43)</td>
<td>73 (71)</td>
<td>48 (47)</td>
<td>0.036</td>
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<tr>
<td>White blood cell count, cells/μL</td>
<td>median (range)</td>
<td>544 (212-2906)</td>
<td>1500 (611-2906)</td>
<td>510 (212-2906)</td>
<td>0.223</td>
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<td>Nucleic acid detection</td>
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<tr>
<td>Plasma HIV RNA viral load, copies/mL</td>
<td>median (IQR)</td>
<td>15 (4-46)</td>
<td>27 (24)</td>
<td>27 (24)</td>
<td>0.006*</td>
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<tr>
<td>CSF beta2-microglobulin, mg/L</td>
<td>median (IQR)</td>
<td>20 (15-30)</td>
<td>25 (25)</td>
<td>25 (25)</td>
<td>0.379</td>
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<tr>
<td>CSF neopterin, nmol/L</td>
<td>median (IQR)</td>
<td>31.7 (19-110)</td>
<td>44 (53)</td>
<td>44 (53)</td>
<td>0.004*</td>
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<tr>
<td>Plasma HIV viral load, copies/mL</td>
<td>median (IQR)</td>
<td>27 (24)</td>
<td>25 (25)</td>
<td>25 (25)</td>
<td>0.379</td>
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<td>ART regimen,</td>
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<td>Pre-exposure prophylaxis (PrEP)</td>
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<tr>
<td>MSM</td>
<td>%</td>
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<tr>
<td></td>
<td>%</td>
<td>44%</td>
<td>44%</td>
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<tr>
<td>Current plasma/HBV/HIV drug mutations</td>
<td>%</td>
<td>31 (27)</td>
<td>31 (27)</td>
<td>31 (27)</td>
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*p-value < 0.05

186 HIV CLINICAL, COMORBID, AND SOCIAL DETERMINANTS OF HEALTH LINKED WITH BRAIN AGING

Kalen J. Petersen, Tina Lu, Julie Wisch, June Roman, Nicolas Metcalf, Sarah Cooley, Beatrice Ours
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Background: Neuroimaging reveals brain changes linked with HIV infection and neurocognitive disorders. However, group-level differences between persons with HIV (PWH) and persons without HIV (PWoH) conceal substantial within-group heterogeneity in risk factor exposures. PWH experience elevated comorbidities such as cardiovascular disease and socioeconomic deprivation. However, the contribution of such factors to brain aging in PWH and PWoH remains to be quantified.

Methods: PWH (n=379; age=44.8±15.5 yr.; 78.1% biologically male; 68.6% African-American; 77.8% undetectable viral load [<50 copies/mL]) and PWoH (n=259; age=38.3±17.1 yr.; 49.8% male; 56.4% A.A.) were clinically characterized and underwent 3-Tesla T1-weighted magnetic resonance imaging (MRI). DeepBrainNet, a publicly available machine learning algorithm, was applied to estimate brain-predicted age from MRI. The brain-age gap (BAG), defined as the difference between brain-predicted age and true chronological age, was modeled as a function of clinical, comorbid, and social factors for PWH and PWoH separately using linear regression and variable selection. To identify spatial patterns relevant to pathological aging, BAG values were correlated with regional brain volumes quantified with FreeSurfer v5.3.

Results: BAG was significantly elevated in PWH compared to PWoH (p<0.001). In PWH, BAG was positively associated with Framingham cardiovascular risk score (p=0.002), detectable viral load (p<0.006) and hepatitis C co-infection (p=0.006). After variable selection, the model for PWH retained Framingham score (p=0.002), detectable viral load (p<0.006) and hepatitis C co-infection (p=0.006). The model for PWoH retained Framingham score (p=0.002). In PWH, BAG was positively associated with Framingham cardiovascular risk score (p<0.006) and hepatitis C co-infection (p=0.006). In PWoH, BAG was positively associated with Framingham cardiovascular risk score (p<0.006) and hepatitis C co-infection (p=0.006).

Conclusion: These findings confirm the hypothesis that comorbid and socioeconomic factors are associated with brain aging alongside clinical metrics such as viral load. A broadened clinical perspective on healthy aging with HIV may require increased focus on such non-traditional determinants of health. Regional correlations between brain-age gap (BAG) and cortical or subcortical volumes.
187 CHANGES TO MICROGLIAL GENOME STRUCTURE AND FUNCTION IN THE HIV-INFECTED HUMAN BRAIN
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Akbarian Lab
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Background: HIV infection of microglia in the central nervous system can lead to HIV-associated neurocognitive disorder (HAND) and contributes to the formation of a potentially large reservoir, but the mechanisms of these processes remain poorly understood. Studies of genome organization and function in the HIV-infected brain are critical to aid in the development of HAND treatments and HIV cure strategies. Here we performed cell-type specific studies of 3D genome architecture, viral integration, and single nucleus transcriptomics in the HIV infected human brain with and without encephalitis.

Methods: Postmortem frontal cortex samples from the Manhattan HIV Brain Bank were processed for 10X Chromium single nucleus RNA-seq (snRNA-seq, n=3 HIV-, n=3 HIV+ without HIV [HIVE], and n=7 HIV+ with HIVE [HIVE]). In situ Hi-C was performed on fixed Irf8+ microglial and NeuN+ neuronal nuclei sorted using fluorescence activated cell sorting (FANS, n=2 HIV-, n=2 HIV+). Integration site sequencing (IS-seq) was performed on FANS isolated NeuN+ neuronal and NeuN- non-neuronal nuclei for a total of 27 samples (n=6 HIV-, n=18 HIV+, n=7 HIV+).

Results: Reorganization of open/repressive (A/B) compartment structures in HIV microglia encompassing 6.4% of the genome was linked to transcriptional activation of interferon (IFN) signaling and cell migratory pathways and was partially recapitulated by IFN-γ stimulation of cultured microglia. In contrast, decreased expression and repressive compartmentalization of genes regulating neuronal health and signaling was seen in both HIV+ and HIVE+ microglia. IS-seq recovered 7,221 integration sites in the brain that displayed distinct genomic patterns as compared to peripheral lymphocyte integration and were enriched for chromosomal domains newly mobilized into a permissive chromatin environment in HIV microglia. Viral transcription occurred in a subset of highly activated microglia comprising 0.003% of all nuclei in HIVE brain.

Conclusion: Our findings point to a disruption of microglia-neuronal interactions in the HIV-infected brain and an interrelation of retroviral integration and expression with interferon-associated remodeling of the microglial 3D genome during progression to HIV. Funded by R01DA054526, and U24MH100931.

188 BRAIN AND BEHAVIOR PREDICTORS OF SARS-CoV-2 INFECTION AMONG PEOPLE WITH HIV
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SEARCH 010/0RV254 Study Team
1University of Missouri St Louis, St Louis, MO, USA, 2SEARCH, Bangkok, Thailand, 3University of Hawaii at Manoa, Honolulu, HI, USA, 4Walton Reed Army Institute of Research, Silver Spring, MD, USA, 5Henry M Jackson Foundation, Bethesda, MD, USA, 6Yale University, New Haven, CT, USA, 7Weill Cornell Medicine, New York, NY, USA, 8University of Miami, Miami, FL, USA, 9University of California San Francisco, San Francisco, CA, USA

Background: Recent findings from the UK Biobank revealed that healthy adults who later became infected with SARS-CoV-2 had lower volumes in regions involved in risk-taking behavior and olfaction compared to individuals who did not become infected. We examined if similar pre-existing differences in brain regions correspond to SARS-CoV-2 infection among people with HIV (PWH) receiving suppressive ART.

Methods: Participants included adult Thai MSM enrolled in the acute HIV (AH1) cohort (RV254/SEARCH010) in Bangkok, Thailand. Participants underwent 3T MRI and clinical assessments (i.e., HIV disease metrics, cognitive testing, and self-reported mood and substance use). ART initiation occurred within 5 days of the MRI (median±same day). Regional brain volumes were summed across hemispheres and corrected for head size. Brain volumes and clinical indices were compared between participants with laboratory confirmed SARS-CoV-2 and those without a diagnosis of SARS-CoV-2 following ART initiation. Machine learning was utilized to identify variables at the time of enrollment into the cohort that predicted subsequent SARS-CoV-2 infection status.

Results: 112 participants were included in the analysis. All study participants achieved viral suppression after ART and received SARS-CoV-2 vaccinations. Fifty-four participants became infected with SARS-CoV-2 during the observation period (median±79 weeks from ART initiation). Study participants who became infected with SARS-CoV-2 after ART had lower volumes at the time of enrollment in several subcortical brain regions with the most pronounced effect in the pallidum (p<0.05). There were no associations between brain volumes and ratings of mood, demographics, or HIV disease indices. SARS-CoV-2 infection was two-fold higher among individuals who reported use of amyl nitrites (i.e., poppers) during “chemsex”. Machine learning with repeated cross validation revealed that lower orbital and medial frontal lobe, anterior cingulate, pallidum, vermis, and olfactory volumes, worse motor function, and higher education collectively predicted co-infection status (average AUC of 85%).

Conclusion: Study findings point toward a risk phenotype for SARS-CoV-2 infection among PWH defined by pre-existing differences in brain volumes relevant to risk-taking behavior, emotion, and neuroHIV as well as behavioral factors such as inhalant use and lack of social distancing during “chemsex”. Machine learning with repeated cross validation revealed that lower orbital and medial frontal lobe, anterior cingulate, pallidum, vermis, and olfactory volumes, worse motor function, and higher education collectively predicted co-infection status (average AUC of 85%).

189 NO CSF BIOMARKER EVIDENCE OF PERSISTING CNS INFECTION AFTER SARS-CoV-2 INFECTION
Martin Stengelin3, George Sigal3, Michael Schöll1, Henrik Zetterberg2, Magnus Gisslen2
1Malmö University Hospital, Malmö, Sweden, 2Messe-Sweden, Stockholm, Sweden, 3Meso Scale Diagnostics, LLC, Rockville, MD, USA

Background: Neurocognitive symptoms are common in acute as well as convalescent (post-acute sequelae of COVID-19 [PASC]) COVID-19, but mechanisms of CNS pathogenesis are unclear. The aim of this study was to investigate cerebrospinal fluid (CSF) biomarker evidence of CNS infection, immune activation and neuronal injury in convalescent compared with acute infection.

Table 1. Demographic and HIV disease indices.

<table>
<thead>
<tr>
<th></th>
<th>Full Sample</th>
<th>HIV+ SARS-CoV-2</th>
<th>HIV- Non-COVID</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>112 (100%)</td>
<td>54 (47%)</td>
<td>58 (53%)</td>
<td></td>
</tr>
<tr>
<td>Age, Mean (SD)</td>
<td>37.4 (10.3)</td>
<td>37.4 (10.5)</td>
<td>37.2 (10.4)</td>
<td>.24</td>
</tr>
<tr>
<td>CDR-1 Total Count, Median (IQR)</td>
<td>324 (248-407)</td>
<td>325 (272-506)</td>
<td>314 (230-417)</td>
<td>.180</td>
</tr>
<tr>
<td>CDR-2 Total Count, Median (IQR)</td>
<td>533 (429-631)</td>
<td>523 (423-786)</td>
<td>540 (513-970)</td>
<td>.802</td>
</tr>
<tr>
<td>CDR-3 Total Count, Median (IQR)</td>
<td>94 (63-165)</td>
<td>96 (48-111)</td>
<td>91 (33-153)</td>
<td>.061</td>
</tr>
<tr>
<td>Plasma Viral Load (log10), Median (IQR)</td>
<td>6.35 (5.63-6.96)</td>
<td>6.14 (5.46-8.67)</td>
<td>6.50 (5.64-6.64)</td>
<td>.087</td>
</tr>
<tr>
<td>Fasting IL-6, s (pg/ml)</td>
<td>32 (28.5)</td>
<td>37 (50.3)</td>
<td>15 (4.6)</td>
<td>.051</td>
</tr>
<tr>
<td>Fasting TNF-α, s (pg/ml)</td>
<td>151 (4.4)</td>
<td>7 (4.2)</td>
<td>25 (4.9)</td>
<td>.046</td>
</tr>
</tbody>
</table>

Results: Fifty-four participants became infected with SARS-CoV-2 during the observation period (median±79 weeks from ART initiation). Study participants who became infected with SARS-CoV-2 after ART had lower volumes at the time of enrollment in several subcortical brain regions with the most pronounced effect in the pallidum (p<0.05). There were no associations between brain volumes and ratings of mood, demographics, or HIV disease indices. SARS-CoV-2 infection was two-fold higher among individuals who reported use of amyl nitrites (i.e., poppers) during “chemsex”. Machine learning with repeated cross validation revealed that lower orbital and medial frontal lobe, anterior cingulate, pallidum, vermis, and olfactory volumes, worse motor function, and higher education collectively predicted co-infection status (average AUC of 85%).

Conclusion: Study findings point toward a risk phenotype for SARS-CoV-2 infection among PWH defined by pre-existing differences in brain volumes relevant to risk-taking behavior, emotion, and neuroHIV as well as behavioral factors such as inhalant use and lack of social distancing during “chemsex”. Machine learning with repeated cross validation revealed that lower orbital and medial frontal lobe, anterior cingulate, pallidum, vermis, and olfactory volumes, worse motor function, and higher education collectively predicted co-infection status (average AUC of 85%).
Methods: We included 68 (35% female) participants ≥18 years of age with CSF sampled during acute (46), 0-3 months after (22) SARS-CoV-2 infection or both (17), and 20 (70% female) healthy controls from longitudinal studies. The 22 patients sampled only at 0-3 months were recruited in a PASC protocol. CSF N-Ag was analyzed using an ultrasensitive antigen capture immunoassay platform (S-PLEX SARS-CoV-2 N Kit, Mesoscale Diagnostics, LLC. Rockville, MD). Additional analyses included CSF β2-microglobulin (β2M), IFN-γ, IL-6, TNF-α, neurofilament light (NFL), and total and phosphorylated tau. Log-transformed CSF biomarkers were compared using ANOVA (Tukey post-hoc test).

Results: Participants sampled during acute infection had moderate (27) or severe (19) COVID-19. In patients sampled at 0-3 months, corresponding initial severity was 10 (mild), 14 (moderate), and 15 (severe). At 0-3 months, 31/39 patients reported neurocognitive symptoms; 8/17 (47%) of patients sampled only at 0-3 months reported neurocognitive symptoms, while 23/39 (60%) of patients sampled at 0-3 months also reported neurocognitive symptoms at 3-6 months. During acute infection, 17/22 patients (77%) had detectable CSF N-Ag, while none were detected at 3-6 months. CSF N-Ag levels during acute infection were significantly higher than at 3-6 months (p<0.0001). CSF N-Ag levels were undetectable in all participants sampled at 3-6 months. CSF N-Ag levels were significantly lower in participants sampled at 3-6 months compared with healthy controls (p<0.0001) and did not differ between the two groups.

Conclusion: The results of this study suggest that CSF N-Ag may be a marker of acute SARS-CoV-2 infection, but its utility as a biomarker of persistent infection or long COVID-19 remains to be determined. Further studies are needed to elucidate the role of CSF N-Ag in the assessment of SARS-CoV-2 infection and persistent CNS damage.

Results from COVID Mind

Participants with new or worsened neurologic symptoms during SARS-CoV-2 infection were enrolled in the COVID Mind Study at Yale. “Never COVID” controls (no history of COVID-19; nucleocapsid (N) negative) were pre-pandemic or prospectively enrolled volunteers. Study at Yale. “Never COVID” controls (no history of COVID-19; nucleocapsid (N) negative) were pre-pandemic or prospectively enrolled volunteers.
Results: Of 670 participants (mITT-E, 447 switched to LA (n=173 [39%] with OLI); n=274 [61%] without OLI) and 223 (33%) continued B/FTC/TAF. Baseline (BL) characteristics were similar between arms; 18% were female sex at birth, 21% were Black, median age (range) was 37 years (18–74). At M11/12, noninferior efficacy of LA vs. B/FTC/TAF was demonstrated for the proportion with HIV-1 RNA ≥50 c/mL (Table). 2.0% and 3.0% participants receiving LA had CVF in the mITT-E and ITT-E populations, respectively; all developed resistance at failure. Excluding injection site reactions (ISRs), AEs and serious AEs were comparable between arms, although drug-related AEs were more frequent in the LA arm (20% vs. <1%). More LA arm participants had AEs leading to withdrawal (6% vs. <1%). Most ISRs were Grade 1 or 2 (98%). Mean adjusted HIV-1 RNA levels scores improved significantly (p<0.001) for LA (+3.36) vs. B/FTC/TAF (−1.59) participants from BL (LA, 57.88; B/FTC/TAF, 58.38; descriptive). At M11/12, most (90%, n=382/425) participants preferred LA vs. oral therapy (5%, n=21/425) at M11/12 or withdrawal.

Conclusion: At M11/12, CAB+RPV LA QD/LA demonstrated noninferior virologic efficacy vs. B/FTC/TAF. Switching to CAB+RPV LA from B/FTC/TAF was efficacious, well tolerated, improved treatment satisfaction, and was preferred by most participants.

**SOLAR Key Efficacy and Safety Outcomes at Month 12**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>QM (n=447)</th>
<th>B/FTC/TAF (n=223)</th>
<th>ITT-E (n=451)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 RNA ≥50 c/mL</td>
<td>2.0%</td>
<td>3.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Grade 3+ AEs</td>
<td>27.7%</td>
<td>25.0%</td>
<td>27.1%</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>8.1%</td>
<td>7.2%</td>
<td>8.1%</td>
</tr>
<tr>
<td>Safety summary</td>
<td>891 AEs</td>
<td>891 AEs</td>
<td>891 AEs</td>
</tr>
</tbody>
</table>

**Key Safety and Efficacy Outcomes**

<table>
<thead>
<tr>
<th>Grade 3+ AEs</th>
<th>B/FTC/TAF (n=223)</th>
<th>ITT-E (n=451)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T-cells</td>
<td>23.9%</td>
<td>24.3%</td>
</tr>
<tr>
<td>CD8+ T-cells</td>
<td>24.4%</td>
<td>23.8%</td>
</tr>
<tr>
<td>B-cells</td>
<td>15.3%</td>
<td>15.1%</td>
</tr>
<tr>
<td>NK-cells</td>
<td>17.2%</td>
<td>17.0%</td>
</tr>
</tbody>
</table>

**EFFECT OF ISLATRAVIR ON TOTAL LYMPHOCYTE AND LYMPHOCYTE SUBSET COUNTS**

**Background:** Islatravir (ISL) is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) being studied for HIV-1 treatment and prevention. Decreases in total lymphocyte counts and CD4+ and CD8+ T-cells were observed in some ISL clinical trials. We conducted comprehensive lymphocyte and lymphocyte subset analyses in Phase 2 and Phase 3 clinical trials evaluating oral ISL with doravirine (DOR) once daily (QD), oral ISL with MK-8507 once weekly (QW), or oral ISL alone once monthly (QM).

**Methods:** These analyses included 4 studies of ISL for HIV-1 treatment: one Phase 2 dose-ranging study of ISL 0.25, 0.75, and 2.25 mg QD with 100 mg (±3TC) in treatment-naive adults (P011), one Phase 2 dose-ranging study of ISL 20 mg with MK-8507 QW in virologically suppressed adults (P013), and two Phase 3 switch studies of DOR/ISL 100 mg/0.75 mg QD in virologically suppressed adults (P017 and P024). For each study, the mean percent change from baseline in total lymphocyte counts and lymphocyte subset counts (CD4+ and CD8+ T-cells, B-cells, and NK-cells) were calculated by treatment group.

**Results:** In treatment-naive participants (P011), increases in total lymphocyte counts (Table) and lymphocyte subset counts were similar for the ISL 0.25-mg group and the DOR/3TC/TDF group and were more favorable than changes observed in the ISL 0.75-mg or 2.25-mg groups. In participants switched to DOR/ISL 100 mg/0.75 mg QD (P017 and P018), decreases in total lymphocyte counts and lymphocyte subset counts reached a nadir on average between Weeks 48 and 72. In participants switched to ISL 20 mg + MK-8507 100, 200, or 400 mg QW (P013) and participants receiving ISL 60 or 120 mg QM for Prep (P016, P022, and P024), decreases in total lymphocyte counts (Table) and lymphocyte subset counts were greater than those in participants receiving DOR/ISL 100 mg/0.75 mg QD (P017 and P018).

**Conclusion:** The magnitude of decreases in total lymphocyte counts and lymphocyte subset counts observed with ISL is exposure-dependent, with the 0.25-mg QD dose having lymphocyte changes comparable to standard of care antiretroviral therapy.

**LENAPACIVIR WITH bNAbs GS-5423 AND GS-2872 DOSED EVERY 6 MONTHS IN PEOPLE WITH HIV**

**Objective:** Lenapacivir (LEN) is a first-in-class HIV-1 capsid inhibitor in development for long-acting HIV treatment and prevention. GS-5423 and GS-2872 are broadly neutralizing antibodies (bNAbs). GS-5423 is derived from 3BNC117 and targets the CD4 binding site of HIV-1 glycoprotein (gp) 120; GS-2872 is derived from 10-1074 and binds to the V3 loop of gp120. Both bNAbs were modified to extend their half-life to allow less frequent dosing. We conducted a phase 1b randomized clinical trial to evaluate the safety and efficacy of LEN + GS-5423 + GS-2872 dosed every 6 months in people with HIV.

**Methods:** Participants were adults living with HIV virologically-suppressed ≥2 years (HIV-1 RNA <50 copies/mL) on ART, sensitive to both bNAbs by HIV proviral DNA phenotyping (PhenoSense mAb IC50 <2ug/mL). In the current study, the primary endpoint was safety; secondary endpoints included virologic outcomes by FDA Snapshot analysis.

**Results:** Of 124 screened participants, 55 were sensitive to both bNAbs, 21 were randomized, and 20 received the complete study regimen. The median age was 44 yrs (IQR 34, 51); 14% were female; 14% Black, 14% Asian, 33% Hispanic/Latinx; median CD4 count was 909 (IQR 687, 1270). There were serious adverse events (AEs), no grade 4 or 5 AEs, and no AEs leading to study drug discontinuation. Two participants had grade 3 AEs: one with injection site cellulitis and one with injection site erythema at the site of LEN injection.
One participant in Group 1 had a confirmed HIV RNA ≥ 50 copies/mL (155 copies/mL, confirmed 524 copies/mL) at Week 16 and resuppressed with reinitiation of baseline ART; one participant in Group 2 withdrew consent at Week 12 (with HIV-1 RNA < 50 copies/mL). 18/20 (90%) participants had HIV-1 RNA < 50 copies/mL at Week 26. (Table 1).

Conclusion: The combination of LEN + GS-5423 + GS-2872 was well-tolerated with high efficacy for 6 months in selected virologically-suppressed persons living with HIV. These results provide a proof-of-concept that this combination could provide long-acting treatment for HIV with twice-yearly dosing.

### Table 1: Efficacy as determined by the US FDA-defined Snapshot Algorithm at Week 26

<table>
<thead>
<tr>
<th>1.0L PN GS-5423 + GS-2872 10 mg/kg</th>
<th>1.0L PN GS-5423 + GS-2872 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 RNA &lt; 50 copies/mL, N 1% [90% CI]</td>
<td>HIV-1 RNA &lt; 50 copies/mL, N 1% [90% CI]</td>
</tr>
<tr>
<td>1 (94.7% [90.1%, 99.4%])</td>
<td>0 (0% [0% , 100%])</td>
</tr>
</tbody>
</table>

### Conclusion: Glecaprevir-pibrentasvir for four weeks was safe and well tolerated among people with acute and recent HCV infection, but efficacy was lower than seen with longer treatment duration (≥ 6 weeks).

### 195 LOW CONCENTRATIONS OF LONG-ACTING CABOTEGRAVIR AND RILPITIVIRINE IN PATIENTS WITH HIV

**Emma Rubenstein,** Myriam Diemer, Laurence Goldwright, Caroline Lascoux, Matthieu Lafaurie, Jermey Zeggagh, Mariagrazia Tateo, Diane Ponsoncare, Julien Gras, Blandine Denis, Agathe Rami, Pierre Sellier, Marie-Laure Chaix, Constance Delauger, Jean-Michel Molina

**University of Paris Cité, Paris, France**

**Background:** Low cabotegravir and rilpivirine trough concentrations have been associated with virological failure in patients with HIV switching to long-acting cabotegravir and rilpivirine in two University Hospitals in Paris. To be eligible, patients had to have a plasma HIV RNA < 50 copies/mL for at least 6 months, no major INSTI or NNRTI resistance-associated mutations and immunity against HBV infection. Intramuscular injections of long-acting cabotegravir 600 mg and rilpivirine 900 mg were administered at the first visit, 4 and 12 weeks later. These first three injections were administered in the hospital as recommended, with monitoring of plasma HIV RNA and trough plasma concentrations of cabotegravir and rilpivirine.

**Results:** Forty-nine patients were enrolled from March to August 2022, 89.8% male, median age 30.3 years. They were mostly men who have sex with men (73.5%), heterosexuals (22.4%), or IV drug user (4.1%) and 6.1% had BMI ≥ 30 kg/m². Median time since HIV diagnosis was 11.4 years. HIV-1 subtypes were B (61.2%), C (4.1%), F (2%), CRF02_AG (16.3%), or other recombinants (14.1%). Median CD4 T-cell count was 680/mm³. With a median time of follow-up of 3.9 months, all patients received two injections, and 38 (84.4%) the Week-12 injection. Median cabotegravir concentration was 972.5 ng/mL (95% CI: 712.2, 749.2) at Week 4, decreasing to 629.5 ng/mL (95% CI: 467.8, 720.3) at Week 12, with respectively 61.2% and 81.1% of patients below the 1120 ng/mL threshold. Median rilpivirine concentration was 49.6 ng/mL (95% CI: 28.6, 66.5) at Week 4, and 43.3 ng/mL (95% CI: 31.6, 53.6) at Week 12, with respectively 26.8% and 27% of patients below the 32 ng/mL threshold. Virological failure without resistance mutation occurred in one patient at Week 4 (plasma HIV RNA: 2820 copies/mL) with cabotegravir and rilpivirine concentrations at 701.3 ng/mL and 27.7 ng/mL respectively at Week 4. This patient had no other risk factor for virological failure.

**Conclusion:** A significant proportion of patients starting long-acting cabotegravir and rilpivirine treatment have low trough concentrations that may increase the risk of virological failure and require careful monitoring.

### 196 SWITCH TO DOR/ISL (100/0.75MG) QD: WEEK 48 RESULTS FROM AN OPEN-LABEL PHASE 3 TRIAL

**Jean-Michel Molina,** 1 Giuliano Rizzardi,2 Catherine Oreill, 3 Alejandro Afani Soud, 1, 4 Alexandra Calmy, 4 Shinichi Oka, 5 Federico Hinestrossa, 2, 6 Princy Kumar, 4 Pablo Tesba, 5 Sharon Walmiskey, 7, 8 Anjana Grandhi, 4, 9 Isaiai Nef Grendrano, 5, 9 Karen Eves, 1, 10 Jason Kim, 1, 10 Todd A. Correll 1

1 University of Paris Cité, Paris, France, 2 Luigi Sacco University Hospital, Milan, Italy, 3 Descend Tutu Health Foundation, Cape Town, South Africa, 4 University of Chile, Santiago, Chile, 5 University of Geneva, Geneva, Switzerland, 6 National Center for Global Health and Medicine, Tokyo, Japan, 7 Orlando Immunology Center, Orlando, FL, USA, 8 Georgetown University, Washington, DC, USA, 9 Hospital of the University of Pennsylvania, Philadelphia, PA, USA, 10 University Health Network, Toronto, ON, Canada, 11 Merck & Co., Inc., Inc., Rathway, NJ, USA

**Background:** Doravirine (DOR), an approved NNRTI, and Islatravir (ISL), an investigational nucleoside reverse transcriptase translocation inhibitor (NRTTI), have complementary mechanisms of action and resistance profiles. We report week 48 results from a phase 3, open-label, non-inferiority trial (P017, NCT04232778) evaluating a switch from oral combination ART to DOR/ISL (100/0.75mg), a once-daily single-tablet regimen.

**Methods:** Virologically suppressed adults on any stable oral 2- or 3-drug ART for ≥3 months with no history of treatment failure or virologic resistance to DOR were randomized (1:1) to switch to DOR/ISL or continue baseline ART (BART), stratified by BART regimen (PI-based, INSTI-based, Other). The primary endpoint was % of participants (pts) with HIV-1 RNA < 50 copies/mL at week 48 (FDA Snapshot; non-inferiority margin 4%). Resistance was assessed in pts with...
clinically significant confirmed viremia (SCV; 2 consecutive measures of HIV-1 RNA ≥200 c/mL).

**Results**: 672 pts were randomized and treated with open-label DOR/ISL (n=336) or B/ART (n=336); 37% were female, 27% Black, mean age 45.5 (±11.7) yrs. B/ART was Pt-based in 14%, inSti-based in 52%, and Other (mainly NNRTI-based) in 34%; mean duration before study 39.4 months. At week 48, 0% on DOR/ISL and 1.3% on B/ART had HIV-1 RNA <50 c/mL (difference 1.5%, 95%CI -3.4, -0.3), while 95.2% and 94.3% respectively had HIV-1 RNA <50 c/mL (Table). SCV occurred in 3 pts (1%) on B/ART (1 inSti-based; 2 NNRTI-based), and had resistance to ≥1 component of their regimen. Mean change from baseline in CD4+ T-cell count at week 48 was 33.0 ± 38.8 cells/mm³ for DOR/ISL and B/ART, respectively (difference 66.7, 95%CI -95.8, -37.7). Total lymphocyte count was also significantly reduced in the DOR/ISL group (Table). Rates of drug-related adverse events (AEs) (19.6%, 8.9%) and discontinuation due to AEs (2.1%, 0.3%) were higher in the DOR/ISL group. Rates of Grade 3-4 AEs (6.8%, 7.4%) and serious AEs (4.2%, 3.9%) were similar between groups. One death occurred (in bART group) due to motor vehicle accident. Infection rates were comparable between treatment groups (33.6% each) and no CDC AIDS-defining Category C events occurred.

**Conclusion**: Switching to DOR/ISL (100/0.75mg) was non-inferior to continuing B/ART for maintaining viral suppression at week 48 and was generally well tolerated. No virologic failure was observed with DOR/ISL. Decreases in CD4+ T-cells and total lymphocytes with DOR/ISL were not associated with differences in infection-related AEs.

**Table. Selected Efficacy and Safety Outcomes, DOR/ISL (100/0.75mg) vs P017**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>DOR/ISL (100/0.75mg)</th>
<th>Baseline ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-RNA &lt;50 c/mL</td>
<td>90.6%</td>
<td>89.7%</td>
</tr>
<tr>
<td>CD4+ T-cell count (cells/mm³)</td>
<td>571.8</td>
<td>707.8</td>
</tr>
<tr>
<td>Mean change from baseline (CD4+ T-cell count)</td>
<td>-0.5 (95% CI -6.9, 5.9)</td>
<td>-27.7 (95% CI -64.3, 29.1)</td>
</tr>
</tbody>
</table>

**198 DZFT: DOLUTEGRAVIR AND DARUNAVIR EVALUATION IN ADULTS FAILING FIRST-LINE HIV THERAPY**

Gail Matthews1, Margaret Borok2, Nekelu Eribo3, Richard Kaplan1, N Kumarasamy4, Ancheale Avihingsanon5, Marco H. Llollo5, Isosker Shah Azwa6, Muhammad Karyana7, Soukouco Do8, Mohamed Cisse9, Emmanuelme Papot10, Simone Jacoby11, Jolie Hutchison12, Matthew G. Law13

**DZFT Study Group**

1University of New South Wales, Sydney, Australia, 2University of Zimbabwe, Harare, Zimbabwe, 3Institute of Human Virology Nigeria, Abuja, Nigeria, 4Desmond Tutu HIV Foundation, Cape Town, South Africa, 5VHS Infectious Diseases Medical Centre, Chennai, India, 6Thai Red Cross AIDS Research Centre, Bangkok, Thailand, 7Hospital JM Ramos Mejia, Buenos Aires, Argentina, 8University of Malaya, Kuala Lumpur, Malaysia, 9National Institute of Health Research and Development, Jakarta, Indonesia, 10University of Bamako, Bamako, Mali, 11Centre de Traitement Ambulatoire de Donka, Conakry, Guinea, 12Kirby Institute, Sydney, Australia

**Background**: Randomised comparative data on efficacy and safety of second-line antiretroviral (ARV) regimens, after failure of non-nucleoside reverse transcriptase inhibitors (NNRTIs) across diverse geographical settings are limited.

**Methods**: DZFT is an international randomised open-label trial comparing dolutegravir (DTG) with ritonavir boosted darunavir (DRV/r) versus DTG with fixed tenofovir and lamivudine or emtricitabine (TDF/FTC) versus standard of care (SOC: DRV/r+2NRTIs with a rotation of nucleosides or in adaptation to HIV genotyping) in adults living with HIV-1 whose first-line NNRTI therapy has failed. The study initially compared DTG+DRV/r vs SOC (stage 1) but later added a third arm (DTG+TDF/FTC, stage 2). Study was designed to show non-inferiority against SOC in terms of HIV-RNA <50 c/mL at 48 weeks using a delta=12% difference from baseline. Primary outcome data are presented as modified intent to treat analysis including all available data.

**Results**: 831 participants from 14 countries across Asia, Africa and Latin America, were randomised: Stage 1 DTG+DRV/r (n=56) vs SOC (n=53); Stage 2 DTG+DRV/r (n=216) vs SOC (n=210) vs DTG+TDF/FTC (n=295). Median age was 55 years and 54% were female. Median CD4 was 206 cells/mm³ and median HIV-RNA was 4.210³ c/mL. First-line failing regimen was efavirenz based in 85%. At 48 weeks the percentage with HIV-RNA <50 c/mL was 75.4% SOC vs 81.4% DTG+DRV/r; in Stage 2: 71.4% SOC, 84.7% DTG+DRV/r, 78.0% DTG+TDF/FTC. Compared to SOC, the % difference in HIV-RNA <50 c/mL was -6.6% (95% CI -12.0, 0.40), demonstrating non-inferiority. HIV-1 RNA was <50 c/mL in 93.8% of participants on DOR/ISL and 94.4% of participants on B/ART (Table). One DOR/ISL participant had CVC at Week 12; ISL plasma concentration was undetectable, and no resistance mutations were identified. Mean change in CD4+ T-cell count was -19.7 and 40.5 (difference -69.1, 95%CI -94.8, -41.4) cells/ mm³, respectively. Total lymphocyte count was also reduced in the DOR/ISL group (Table). Rates of AEs (71.1%, 74.6%) and drug-related AEs (9.9%, 11.9%) were similar between groups, with low rates of discontinuation due to AEs (both 2.5%). The most common AE in both groups was headache (7.8%, 7.2%). Infection rates were comparable between treatment groups (31.4%, 30.7%) and there was 1 CDC AIDS-Defining Category C event in each group (esophageal candidiasis in DOR/ISL, recurrent Kaposi sarcoma in B/ART).

**Conclusion**: Switching to DOR/ISL (100/0.75mg) was non-inferior to continuing B/ART for maintaining viral suppression through Week 48 with an AE profile comparable to B/ART. No treatment-emergent resistance was detected in the DOR/ISL group. Observed decreases in CD4+ T-cells and total lymphocytes on DOR/ISL were not associated with differences in infection-related AEs.

**Table. Selected Efficacy and Safety Outcomes, DOR/ISL (100/0.75mg) vs P018**

**Week 48 Virological Outcomes, n (%)**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>DOR/ISL (100/0.75mg)</th>
<th>Baseline ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-RNA &lt;50 c/mL</td>
<td>94.1%</td>
<td>94.3%</td>
</tr>
<tr>
<td>Mean change from baseline (HIV-RNA)</td>
<td>-0.7 (95% CI -3.4, 2.0)</td>
<td>1.7 (95% CI -8.9, 11.3)</td>
</tr>
</tbody>
</table>

**Week 48 Adverse Events, n (%)**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>DOR/ISL (100/0.75mg)</th>
<th>Baseline ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-related AEs</td>
<td>29.3%</td>
<td>29.3%</td>
</tr>
<tr>
<td>Mean change from baseline (CD4+ T-cell count)</td>
<td>-19.7 (95% CI -31.4, -8.1)</td>
<td>8.7 (95% CI 1.4, 16.0)</td>
</tr>
<tr>
<td>Mean change from baseline (CD4+ T-cell count)</td>
<td>-20.2 (95% CI -28.5, -11.9)</td>
<td>0.5 (95% CI 1.5, 3.5)</td>
</tr>
<tr>
<td>Mean change from baseline (CD4+ T-cell count)</td>
<td>0.4 (95% CI 1.1, 0.6)</td>
<td>3.7 (95% CI 3.1, 4.3)</td>
</tr>
</tbody>
</table>

1 Data as observed approach. The within-group 95% CIs were calculated based on the distribution of the t-distribution.
HIV RECENTY TESTING WITH INDEX TESTING CAN IDENTIFY NEW INFECTIONS EARLIER IN RWANDA
Suzue Saito, Giles Reid, Eugenie Poirot, Jean-Claude Iribona, Collins Kamaruzi, Veronica Migaba, Beata Sangwireyi, Eric Remera, Tom Olouch, Richard Mwesigwa, Elysee Tuyishime, Kemba Lee, David A. Miller, Amitabh B. Suthar, Gallician N. Rwibasa
Rwanda HIV Recency Evaluation Study Team
1ICAP at Columbia University, New York, NY, USA, 1ICAP at Columbia University, Kigali, Rwanda, 2Centers for Disease Control and Prevention, Kigali, Rwanda, 3Rwanda Biomedical Centre, Gasabo, Rwanda, 4Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: In 2018, Rwanda implemented HIV recency testing as part of active case surveillance and index testing to inform prevention strategies. Recency testing can shed light on transmission dynamics by helping to distinguish recent and long-term (LT) infections among newly diagnosed people. We examined yields of HIV positivity and HIV recency among sexual partners of newly diagnosed people in Rwanda.

Methods: We conducted a prospective cohort study of 1,238 newly diagnosed people undergoing recency and index testing at 60 health facilities selected using probability sampling in all five provinces in Rwanda from August 2021–October 2022. We classified recent and LT infections based on a rapid test for HIV recency (RTRI) followed by baseline viral load (VL). RTRI LT with a VL of ≥1000 copies/mL were classified as a recent case. We compared the average number of sexual contacts elicited, and HIV positivity and HIV recency yields among the sexual contacts of recent vs. LT cases over a 6-month period using Fisher’s exact test. HIV positivity yield was defined as the number of new HIV positive sexual contacts identified among all sexual contacts tested for HIV. HIV recency yield was defined as the number of sexual contacts with a recent infection identified among all sexual contacts tested for HIV.

Results: We enrolled 98 recent and 1,140 LT newly diagnosed people who identified 1,738 sexual contacts as part of index testing. Index cases had an average of 6.6 clinic visits during study follow up. Recent index cases were more likely to be female compared to LT cases (79% vs. 62%), under 35 years of age (72% vs. 60%), single (38% vs. 30%), and men who have sex with men or female sex workers (22% vs. 9%) (p<0.01 for all comparisons). For every recent index case, 1.67 sexual contacts were elicited compared to 1.38 contacts per LT case (p=0.06). HIV positivity yield among sexual contacts of recent and LT index cases were 20% and 15%, respectively (p=0.3) while HIV recency yield was 4% vs. 0.8% (p=0.045).

Conclusion: Newly diagnosed people with recent infections compared to those with LT infections were associated with more sexual contacts with recent infections. HIV recency with index testing provides an important opportunity to identify new infections earlier and timely offer prevention resources to curb onward transmission.
THE IMPACT OF PATIENT-CENTRED CARE ON HIV TREATMENT IN ZAMBIA: A STEPPED-WEDGE TRIAL

Kombatende Sikombe1, Aaloke Mody2, Charles Goss3, Ingrid Eshun-Wilson4, Sandra Simbeza5, Anjali Sharma1, Laura K. Beres4, Jake M. Pry5, Njekwa Mukamba1, Brian Rice1, Jacob Mutale6, Carolyn Bolton Moore1, Charles B. Holmes3, Izukani Sikazwe7, Elvin Geng2

1Center for Infectious Disease Research in Zambia, Lusaka, Zambia, 2Washington University in St Louis, St Louis, MO, USA, 3Monterrey Medical Center, Bronx, NY, USA, 4The Johns Hopkins University, Baltimore, MD, USA, 5London School of Hygiene & Tropical Medicine, London, United Kingdom, 6Georgetown University, Washington, DC, USA

Background: Patient-providers interactions drive HIV-care retention. How to change HCW attitudes, communication, and practice is poorly understood. We evaluated a multicomponent patient centred care (PCC) intervention comprising HCW training, practice facilitation, and incentives, to improve patient-experience in Zambia.

Methods: 24 clinics in Lusaka, Zambia, participated in a stepped wedge trial (August 2019–November 2021), over approximately six month periods. Treatment failure (HIV RNA > 400 copies/ml or disengagement) was our primary outcome at 15 months. Secondary outcomes were retention at 15 months (i.e., a visit between 11 and 19 months) in waves one and four, for an appointment (>30 days) in all waves, and trained patient experience survey (providers were blinded). The intervention included 1) systematic measurement and response to patient experience (satisfaction, HCW attitude/communication, timeliness), 2) PCC training and mentoring and 3) small incentives to enhance performance change HCW attitudes, communication, and practice is poorly understood. We evaluated a multicomponent patient centred care (PCC) intervention comprising HCW training, practice facilitation, and incentives, to improve patient-experience in Zambia.

Results: 933 (58% female, median age 37 (IQR:30–44) participants were recruited in 16 facilities for our primary outcome. We found no difference in treatment-failure between control (N=453) and intervention (N=480) (RD=0.9%, 95% CI: -0.3 - 9.7) [wave 1 and 4, N=84,926], this effect was greatest among reengaged (RD 5.2% [95% CI: 0.1 - 10.3], N=17,276) and new ART patients (RD 11.3% [95% CI: 0.2 - 22.5], N=8,817). Intervention patients missed fewer visits than control visits (waves 1 and 4, RD =3.3% [95% CI: -3.7 - 2.9]), N=1,087,809 visits). The effect on missed visits was greatest among new ART patients (RD 5.5% [95% CI: -7.3 - 2.6], N=54,886 visits). After 6 months of intervention, patient experience improved considerably (Sum score mean, 0.85; 95% CI: 0.37 - 1.32).

Conclusion: A multi-component, participatory intervention improved patient experience and retention, but not treatment-success. Improving patient experience through PCC practice facilitation can complement differentiated HIV care delivery techniques.

A RANDOMIZED CONTROL TRIAL OF MHEALTH TOOL EFFECT ON VIRAL SUPPRESSION IN YOUTHS

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1Makerere University College of Health Sciences, Kampala, Uganda, 2Infectious Diseases Institute, Kampala, Uganda

Background: Adherence to antiretroviral therapy (ART) is the principal determinant for achieving and sustaining viral suppression, which decreases progression to AIDS, and reduces mortality and transmission risk. Whilst burden of HIV is increasing in adolescents and youth, adherence to medications, retention in care and self-management is still a challenge. It is important to evaluate if the emerging capacity in mHealth technology could support youth living with HIV. We evaluated whether Call for life-interactive voice response (CFL-IVR, Johnson & Johnson) mHealth tool improves ART adherence and retention in care among youth receiving ART at a rural district in Uganda.

Methods: This was a randomized trial in a rural Ugandan district, 225 KM away from the capital. Recruitment was from three ART clinics, youth living with HIV 15-24 years, initiating or on ART for ≤ 6 months were eligible for randomization at a ratio of 1:1 to either Standard of Care (SoC) which is usual care or the intervention “CFL-IVR plus SoC”. Participants were seen at baseline, month 6, and month 12. Data collection included socio-demographics, medical history, HIV basic Knowledge, and sexual behaviors. Blood for viral load testing was drawn at ≥3 clinic visits. Data was entered in REDCap and exported to stata 15. The CFL-IVR tool offered; individualized pill reminders, clinic visit reminders, health tips messages, and functionality to support self-reported symptoms. Descriptive and regression analysis were done to identify factors related to ART adherence, viral suppression and retention in care.

Results: We randomized 206 participants in a ratio 1:1 to either CFL-IVR intervention or SoC. Participants mean age were 22.3yrs, 167 (81%) were females, 183 (88%) had a sexual partner, 174 (84%) were working, 63 (30%) had monthly household income of at least 26 USD and 52 (25%) had secondary level of education. Retention in care at month 6 was 93.2% in intervention vs 84.3% in SoC (p=0.046) and at 12 months 94.8% in intervention vs 89.5% in SoC (p=0.122). Viral suppression: At 12 months, 67.4% (60) of those on intervention had a suppressed VL compared to 54.7% (41) SoC, which was statistically significant p=0.009. Factors related to viral suppression were age above 22yrs, being female, and having a partner (Table 1: Factors associated with VLS). mHealth has demonstrated significant potential to improve medication adherence and retention in care.

Table of factors associated with viral load suppression at 12 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 22yrs</td>
<td>0.977</td>
<td>0.900 - 0.999</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.380</td>
<td>0.990 - 1.920</td>
<td></td>
</tr>
<tr>
<td>Having a partner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.130</td>
<td>0.950 - 1.330</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detectable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppressed</td>
<td>0.253</td>
<td>0.200 - 0.320</td>
<td></td>
</tr>
<tr>
<td>simply_forgot Pills</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.179</td>
<td>1.000 - 1.390</td>
<td></td>
</tr>
<tr>
<td>Fear of rejection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.682</td>
<td>0.491 - 0.947</td>
<td></td>
</tr>
</tbody>
</table>

203 SUPPRESSING VIRAL LOAD IN PEOPLE WITH HIV WHO INJECT DRUGS IN RUSSIA: LINC-IIRCT

Jeffrey H Samet1, Elena Blokhina2, Debbie M. Cheng3, Sydney Rosen4, Dmitry Lianzov5, Karsten Lunze6, Ye Truong6, Natalia Gnatienko5, Natalia Bushara7, Anita Raj8, Evgeny Krupitsky9

1Boston University, Boston, MA, USA, 2First Pavlov State Medical University of St Petersburg, Saint Petersburg, Russia, 3Boston Medical Center, Boston, MA, USA, 4University of California San Diego, San Diego, CA, USA

Background: Over 1.4 million people with HIV (PWH) live in Russia; only 56% are on antiretroviral therapy (ART). Substance use is associated with delayed linkage to HIV care and suboptimal use of ART. In Russia, routine HIV testing is the norm in addiction treatment systems, but if positive, linkage to HIV care is limited.

Methods: We conducted a two-arm, randomized controlled trial in St. Petersburg assessing the effectiveness of a multi-faceted intervention (rapid access to ART while admitted to an addiction hospital, provision of depot naltrexone for opioid use disorder, and 12-months of strengths-based case management) compared to usual care. Study eligibility included: PWH, past drug injection, currently not on ART, and an addiction hospital inpatient.
The primary outcome was undetectable HIV viral load (HVL) at 12 months. Secondary outcomes included undetectable HVL at 6 months, initiation of ART ≤28 days of randomization, retention in HIV care at 12 months, change in CD4 count, and a composite outcome of HVL suppression and past 30-day opioid abstinence. We performed adjusted logistic and linear regression analyses controlling for past ART use using an intention-to-treat approach.

**Results:** Among 225 (N=111 intervention; N=114 control) participants (60% male, mean age 37, mean baseline CD4 count 420 cells/mm3) nearly all (99%) had severe opioid use disorder, 33% reported previous ART use, and 76% had depressive symptoms. Compared to the control group, intervention participants had higher odds of achieving the primary outcome, undetectable HVL at 12 months (48% intervention vs. 22% controls; adjusted odds ratio [AOR] 3.04; 95% confidence interval [CI]: 1.44, 6.44; p=0.004). Secondary outcomes were undetectable HVL at 6 months (35% vs 13%; AOR 3.76; CI: 1.7, 8.34; p=0.001), initiating ART within 28 days of randomization (74% vs. 11%; AOR 23.13; CI: 11.13, 48.07; p<0.001), retention in HIV care at 12 months (51% vs. 35%; AOR 1.97; CI: 1.15, 3.37; p=0.014), change in CD4 counts (+59 vs. 0; adjusted mean difference 63.95; CI: -2.36, 130.25; p=0.059), and achieving the composite outcome HVL suppression and past 30-day opioid abstinence at 12 months (27% vs. 5%; AOR 6.51; CI: 2.08, 20.40; p=0.001).

**Conclusion:** Among PWH who inject drugs in Russia, a multi-faceted intervention combining rapid, in-hospital ART initiation, naltrexone, and case management was more effective than standard of care for achieving undetectable HVL, initiating ART, retention in HIV care, and reducing opioid use. Effect of LINC II intervention compared with standard of care and HIV substance use outcomes, adjusted for history of ART use.

**204 URINE TENOFOVIR LEVELS PREDICT VIRAL SUPPRESSION IN PATIENTS ON TENOFOVIR ALAFENAMIDE**

**Kelly A. Johnson, Okochi Hideaki, Mineya Arreguin, Joseph Watabe, David V. Gliddon, Anindita Chattopadhyay, Elizabeth Imbert, Matthew D. Hickey, Monica Gandhi, Matthew A. Spinelli
University of California San Francisco, San Francisco, CA, USA**

**Background:** We previously developed a point-of-care (POC) assay to measure urine tenofovir (TFV) levels among patients on tenofovir disoproxil fumarate (TDF), with a cut-off of 1500 ng/ml indicating adequate adherence. Since tenofovir alafenamide (TAF) results in lower plasma concentrations than TDF, the clinical utility of the existing assay for patients on TAF is unknown. We thus explored whether urine TFV levels above the 1500 ng/ml cut-off correlate with future virologic suppression (VS) (< 200 copies/mL) among people living with HIV (PLWH) on TAF.

**Methods:** We collected urine samples from patients with HIV on TAF-based regimens at two San Francisco HIV primary care clinics from 06/2019-12/2021. We used our electronic medical record to describe sociodemographic/clinical characteristics, then measured continuous urine TFV levels via liquid chromatography/tandem mass spectrometry. Finally, we used generalized estimating equations to fit a model comparing urine TFV levels (above and below 1500 ng/ml) for samples with and without VS on the first viral load measured after urine collection.

**Results:** Our analysis included 83 samples (68 suppressed and 15 unsuppressed) from 67 unique PLWH. Samples from person-visits with/without VS were similar in age (median 55 vs 45 years), sex (88% vs 100% male), gender (75% vs 67% cis-male), race/ethnicity (46% vs 33% Black; 37% vs 33% White), glomerular filtration rate (GFR, 84% vs 87% >60 ml/min), creatinine (1.02 vs 0.85 mg/dL), and weight (81.6 vs 73.7 kg); all with p >0.05. The median (interquartile range) urine TFV levels by LC-MS/MS were respectively 3190 (1460, 5720) ng/mL in samples from visits with VS and 690 (70, 2100) ng/mL from visits without suppression. In adjusted modeling accounting for age, race/ethnicity, and GFR (Table 1), urine TFV levels >1500 ng/ml were strongly associated with future VS (OR 5.66; 95% CI 1.59-20.14; p=0.007). The sensitivity/specificity/PPV/ NPV at the 1500 ng/ml cut-off were respectively 75%, 67%, 91%, and 63%.

**Conclusion:** Urine TFV levels above 1500 ng/ml were strongly predictive of future VS among patients on TAF, suggesting that individuals taking TAF who flag below 1500 ng/ml on POC testing would benefit from enhanced adherence counseling. Our results further imply: (1) that the existing POC assay originally developed for TDF may have real-world applicability to predict VS among people with HIV on TAF, and (2) that a single POC assay may support adherence monitoring for patients on both TAF and TDF worldwide.

### Table: Adjusted Generalized Estimating Equations Model for Future Virologic Suppression by Urine Tenofovir (TFV) Levels (above 1500 ng/ml) among People with HIV on TAF-based Antiretroviral Therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine TFV level above 1500 ng/ml by LC-MS/MS</td>
<td>5.66 (1.59-20.14)</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (per ten year increase)</td>
<td>0.90 (0.25-3.21)</td>
<td>0.71</td>
</tr>
<tr>
<td>Race Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.09 (0.68-1.73)</td>
<td>0.97</td>
</tr>
<tr>
<td>Latina</td>
<td>0.28 (0.09-0.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>Other Race/Missing</td>
<td>1.04 (0.45-2.45)</td>
<td>0.93</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>0.86 (0.75-0.98)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**205 TENOFOVIR DIPHOSPHATE IN DRIED BLOOD SPOTS AND HIV-1 RESISTANCE IN SOUTH AFRICA**

**Yashna Singh¹, Jose Castillo-Mancilla², Lauren Jennings¹, Richard Madimabe¹, Christopher Ferrari³, Reuben N. Robbin⁴, Peter L. Anderson⁴, Robert H. Remien⁵, Catherine Orrell⁶
¹ Desmond Tutu Health Foundation, Cape Town, South Africa, ²University of Colorado Denver, Denver, CO, USA, ³Columbia University, New York, NY USA, ⁴New York State Psychiatric Institute, New York, NY, USA, ⁵University of Colorado Anschutz Medical Campus, Aurora, CO, USA**

**Background:** Suboptimal antiretroviral (ART) adherence can lead to virologic failure with consequent HIV-1 resistance. Tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) is a powerful biomarker of cumulative ART adherence that is predictive of present and future viremia in persons with HIV (PWH) in South Africa (SA) and the US. This study explored the relationship of TFV-DP concentrations with antiretroviral drug resistance at the time of treatment failure in SA.

**Methods:** Adult PWH from health clinics in Cape Town, SA on efavirenz-based first-line ART containing tenofovir disoproxil fumarate, who had an undetectable (< 50 copies/mL) HIV viral load (VL), were prospectively enrolled in an observational cohort for 12 months. Monthly study visits included blood collection for HIV VL and DBS for TFV-DP. Viral breakthrough (VB) was defined as the first confirmed HIV VL > 400 copies/mL, and HIV-1 genotyping was completed at the next visit after VB. ART adherence was monitored using an electronic adherence (EA) monitor, and estimated as a percent for the 30-days prior to VB. Non-parametric statistics were used to compare median (IQR) TDF-DP by genotype outcome.

**Results:** Of 250 participants (n=195, 78% women), 21 experienced VB, with a median (IQR) of 5 (4-7) months on study, and a median EA of 33.3 (13.5,33.3) %.

There were no demographic differences between those with and without VB. Median (IQR) VL at VB was 4.0 (3.2-4.5) log copies/mL. TFV-DP concentrations trended downwards towards the VB visit (Figure). Median (IQR) TDF-DP concentrations were compared (p=0.035) and were significantly higher those whose HIV-1 genotype did not amplify due to being virally suppressed at the subsequent visit (n=10; 380 [227-661] fmol/punch, ; EA 45 [24.9; 59.2]%); whose HIV-1 genotype did not amplify due to being virally suppressed at the subsequent visit (n=10; 380 [227-661] fmol/punch, ; EA 45 [24.9; 59.2]%); whose HIV-1 genotype did not amplify due to being virally suppressed at the subsequent visit (n=10; 380 [227-661] fmol/punch, ; EA 45 [24.9; 59.2]%); whereas those whose HIV-1 genotype did not amplify due to being virally suppressed at the subsequent visit (n=10; 380 [227-661] fmol/punch, ; EA 45 [24.9; 59.2]%) did not have detectable ART adherence at the time of treatment failure in SA.

**Conclusion:** TFV-DP in DBS showed a step-wise inverse relationship with VB and drug resistance, with evidence of low (but not completely absent) cumulative low ART adherence in PWH who developed antiretroviral resistance.
ART SHARING IS COMMON AND ASSOCIATED WITH VIREMIA: A POPULATION-BASED STUDY IN UGANDA
Caitlin E. Kennedy1, Xin Yi Feng2, Joseph G. Rosen3, Joseph Sekasanvu1, Robert Ssekubugu1, Godfrey Ngiga1, David M. Serwadda4, Joseph Kabanda1, Pingo Teresa Yeh1, Joseph Kagaayi2, Steven J. Reynolds2, Larry William W. Chang2, Fred Nalugoda1, Mary K. Grabowski2
The Rakai Health Sciences Program
1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3Rakai Health Sciences Program, Entebbe, Uganda, 4Chinese Center for Disease Control and Prevention, Kampala, Uganda

Background: Antiretroviral treatment (ART) sharing has been reported among fisherfolk and sex workers in Uganda and South Africa. However, to date, no population-based studies have documented the prevalence of ART diversion (including sharing [giving or receiving], buying, and selling ART pills) among men and women living with HIV in Africa, or its relationship with HIV viremia in these groups.

Methods: From 2018-2020, we surveyed all people living with HIV aged 15-49 in 40 communities in the Rakai Community Cohort Study (RCCS), a population-based cohort in south-central Uganda. We assessed prevalence and correlates of self-reported ever and past-year ART diversion. We stratified data by age and gender, and documented diversion partners. We used log-binomial regression to assess the relationship between diversion patterns and viremia (viral load >1000 copies/ml), reported as risk ratios (RR) with 95% confidence intervals (CI).

Results: Of 2,852 people living with HIV, 266 (9.3%) reported ART diversion. Giving and receiving were most common: 62 (2.2%) reported giving only, 54 (1.9%) reported receiving only, and 132 (4.6%) reported both giving and receiving. Few participants reported buying (n=18, 0.6%) and none reported selling. Men were more likely to report any diversion than women (12.9% vs. 7.4%), with men aged 25-34 reporting particularly high levels of sharing (18.9%) (Figure). Friends were the most common sharing partners, followed by spouses/sexual partners. Patterns of ever and past-year diversion were similar for age and gender. Among participants with viral load results, 8.6% (234/2,725) were viremic. People who reported only giving ART to others were twice as likely to be viremic than those who reported no diversion (RR: 2.04, 95% CI: 1.14-3.63), while those who reported only receiving ART had a non-significant lower prevalence of viremia (RR: 0.48, 95% CI: 0.12-1.89). Reporting both giving and receiving was not associated with viremia (RR: 0.90, 95% CI: 0.49-1.66).

Conclusion: This first population-based assessment of ART diversion found that ART sharing is fairly common in rural Uganda, particularly among men. While receiving pills may support viral suppression, giving pills is associated with viremia. HIV programs may benefit from considering drug sharing in counseling messaging. Future longitudinal studies are needed to assess temporal relationships.

EFFECTIVENESS OF SMALLPOX VACCINATION TO PREVENT MPOX IN MILITARY PERSONNEL
Boghuma K. Titanji1, Angela Eick-Cost2, Lauren Epstein3, Natalie Welsi4, Jeremy Smith5, Shauna L. Stahlman6, Abirami Balajee7, Saiju Pyarajan8, Chris W. Woods6, Mark Holodnij9, Elizabeth J. Partan1, Victoria J. Davey1, Robert A. Bonomo1, Yinong Young-Xu1, Vincent C. Marcon1
1Emory University, Atlanta, GA, USA, 2Armed Forces Health Surveillance Division, Defense Health Agency, U.S. Department of Defense, Silver Spring, Maryland, USA, 3Silver Spring, MD, USA, 4Emory University School of Medicine, Atlanta, GA, USA, 5White River Junction Veterans Affairs Medical Center, White River Junction, VT, USA, 6VA Center for Data and Computational Sciences, Boston, MA, USA, 7Duke University, Durham, NC, USA, 8Stanford University, Los Angeles, CA, USA, 9US Department of Veterans Affairs, Washington, DC, USA, 10Case Western Reserve University School of Medicine, Cleveland, OH, USA

Background: The effectiveness of Smallpox vaccines against Mpox is unknown. In the global outbreak of Mpox, these vaccines are being used as prevention in individuals with high-risk of exposure to Mpox. There is an urgent need to determine their effectiveness.

Methods: We conducted a retrospective, test-negative case-control study among US current and former military personnel. The goal of the study was to evaluate the vaccine effectiveness (VE) of smallpox vaccines against Mpox. Uni- and multivariate logistic regressions were used to calculate odds ratios (ORs) with 95% confidence interval (CI) for the association between positive Mpox testing and receipt of Smallpox vaccine. Vaccine effectiveness (VE) was estimated as (1− OR of Mpox in vaccinated/OR of Mpox in unvaccinated) x 100%. Models for adjusted analyses included covariates for potential confounders of the association between vaccination status and testing positive for Mpox. The adjustment covariates included age, race, sex, and HIV status. Among individuals tested in the VA system, severity of disease was defined by admission to the hospital for Mpox. This information was not available for individuals outside of a VA facility. Analyses were conducted using Stata 17 and SA59.4 software.

Results: The analysis included 959 military personnel and Veterans who were tested for Orthopoxvirus between July 1-October 31, 2022. 188 (19.6%) of whom had a documented Smallpox vaccination. Among the 290 individuals who tested positive for Orthopoxvirus, 24 (8.3%) were previously vaccinated with ACAM2000 (“2nd-generation” Smallpox vaccine) and 12 (4.1%) were previously vaccinated with Variola vaccinia among military personnel.
vaccinated with Dryvax (“1st-generation” Smallpox vaccine). The median time from receipt of Smallpox vaccination to Mpx diagnosis was 12-years (range: 35, IQR:6.5). Individuals with prior ACAM2000 (OR=0.28; 95%CI: 0.17-0.47) or Dryvax (OR=0.36; 95%CI: 0.18-0.73) vaccination were less likely to test positive for Orthopoxvirus compared to unvaccinated individuals. The estimated VE rate was 72% for ACAM2000 and 64% for Dryvax. Among those who tested positive for orthopoxvirus within the VA, 14/183 (7.7%) required hospitalization; Of those who tested positive, 126 (43.4%) were people with HIV (OR: 2.30; 95% CI: 1.63 – 3.25)

Conclusion: Vaccination with Smallpox vaccines reduces the likelihood of testing positive for Orthopoxvirus. Our study provides the largest evaluation of the effectiveness of earlier smallpox vaccines against Mpx, supporting their usefulness in containing the global outbreak.

Table 1: Demographic and clinical characteristics of individuals tested for non-vaccine Orthopoxvirus (NPOX) across EEU and VA

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Number Tested (N)</th>
<th>Number Positive (N)</th>
<th>Positive Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACAM2000</td>
<td>183</td>
<td>14</td>
<td>7.7</td>
</tr>
<tr>
<td>Dryvax</td>
<td>183</td>
<td>126</td>
<td>64.2</td>
</tr>
</tbody>
</table>

At the time of Mpx outbreak, 546 MSM had been randomized to the study. We assessed the impact of vaccination on incidence of Mpx on PrEP participating in a clinical trial.

Methods: The ANRS-174 DOXYVAC trial enrolled 546 MSM for vaccination with multiple-partner MSM was recommended as of July 11, 2022. We assessed the impact of vaccination on incidence of Mpx on PrEP participating in a clinical trial.

Results: We identified 129 pediatric household contacts exposed by 79 adult index cases. The median age of contacts was 7 years (range 0-15 years). Eighty children (14%) exposed by 14 adult index cases reported symptoms consistent with Mpx during their incubation period; their median age was 6 years (range 1-11 years). All symptomatic children reported a rash; other symptoms included adenopathy, fever, fatigue, and myalgia. Among 18 symptomatic contacts, 12 (66.7%) underwent Mpx testing; 5 (41.7%) were confirmed cases, 6 (50%) were negative, and 1 (0.8%) had an indeterminate result. Two of the confirmed infected pediatric contacts came from a single household (Household 1). Six symptomatically ill children were not tested for Mpx (33.3%). One of these children, also a resident of Household 1, was considered to be a probable case due to consistent rash and systemic symptoms. Overall, six infected contacts were identified through August 2022, resulting in a SAR of 4.7% (6 of 129). Among infected contacts, median age was 4.5 years (range 2-9 years) and three of six resided in the same household (Household 1).

Conclusion: In France, MVA-BN vaccination in summer 2022 conferred high-level protection against Mpx infection in highly-at-risk MSM on PrEP. In this study population, sexual behavior change did not seem to play a role in reduction of Mpx incidence.

Table: Poisson regression model

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<th>Variable</th>
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<tr>
<td>Period</td>
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208 IMPACT OF VACCINATION ON MPOX INCIDENCE IN MSM ON PRPT IN THE ANRS 174 DOXYVAC TRL

Jade Ghose1, Lambert Assoumou2, Moussa Ouattara3, Michele Algarte-Gerin4, Emma Rubenstein5, Gilles Pialoux6, Christine Katlama7, Laure Surgers8, Claudine Duvivier9, Juliette Pavie10, Jean-Paul Viard11, Severine Giboiski12, Manon Ollivier12, Dominique Costagliola13, Jean-Michel Molina14

1University of Paris Cité, Paris, France, 2Sorbonne Université, Paris, France, 3Institut National de la Santé et de la Recherche Médicale, Chilly, France, 4Hôpital-Dieu de Paris, Paris, France, 5ANRS | Emerging Infectious Diseases, Paris, France

Background: Mpx was first reported in France on May 19, 2022 and third-generation live Modified Vaccinia Ankara (MVA-BN) vaccination of multiple-partner MSM was recommended as of July 11, 2022. We assessed the impact on incidence of Mpx in MSM on PrEP participating in a clinical trial.

Methods: The ANRS-174 DOXYVAC trial enrolled 546 MSM for vaccination with multiple-partner MSM was recommended as of July 11, 2022. We assessed the impact on incidence of Mpx in MSM on PrEP participating in a clinical trial.

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Conclusion: In France, MVA-BN vaccination in summer 2022 conferred high-level protection against Mpx infection in highly-at-risk MSM on PrEP. In this study population, sexual behavior change did not seem to play a role in reduction of Mpx incidence.

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209 HOUSEHOLD TRANSMISSION OF MPOX TO CHILDREN AND ADOLESCENTS

Darpan Sachdev1, Rilene Ng1, Cameron Steinken1, Meredith Haddix1, Erin Peterson2, Kristen Wendorf2

1California Department of Public Health, San Francisco, CA, USA, 2California Department of Public Health, Richmond, CA, USA, "Los Angeles County Department of Public Health, Los Angeles, CA, USA

Background: In California, 5572 cases of Monkeypox (MPOX) were identified in California as of November 28, 2022, approximately 20% of the US case count; 0.3% of cases were among children < 16 years old. We analyzed routinely collected contact tracing data in California to assess the secondary attack rates (SAR) among pediatric household contacts exposed to MPOX.

Methods: Using data from California’s public health database, and data separately provided by San Diego and Los Angeles counties, we created a list of all pediatric MPOX contacts aged < 16 years reported through August 31, 2022. Contacts to a non-confirmed or probable case of MPOX and non-household contacts were excluded from analysis. All pediatric contacts were reviewed to ascertain whether symptoms developed during their incubation period (21 days after last exposure) and outcomes of testing; pediatric contacts that did not report symptoms were assumed to have been asymptomatic.

Results: We identified 129 pediatric household contacts exposed by 79 adult index cases. The median age of contacts was 7 years (range 0-15 years). Eighteen children (14%) exposed by 14 adult index cases reported symptoms consistent with MPOX during their incubation period; their median age was 6 years (range 1-11 years). All symptomatic children reported a rash; other symptoms included adenopathy, fever, fatigue, and myalgia. Among 18 symptomatic contacts, 12 (66.7%) underwent MPOX testing; 5 (41.7%) were confirmed cases, 6 (50%) were negative, and 1 (0.8%) had an indeterminate result. Two of the confirmed infected pediatric contacts came from a single household (Household 1). Six symptomatically ill children were not tested for MPOX (33.3%). One of these children, also a resident of Household 1, was considered to be a probable case due to consistent rash and systemic symptoms. Overall, six infected contacts were identified through August 2022, resulting in a SAR of 4.7% (6 of 129). Among infected contacts, median age was 4.5 years (range 2-9 years) and three of six resided in the same household (Household 1).

Conclusion: Among children with household contact to an adult with MPOX in California, only 14% developed symptoms consistent with MPOX, and less than 5% ultimately tested positive. The secondary attack rate may have been underestimated because one-third of symptomatic children were not tested. While the risk of household transmission is low, pediatric household contacts should be offered post-exposure prophylaxis to prevent MPOX spread.
210 ASSOCIATION BETWEEN OBSERVED MASKING AND SARS-CoV-2 PRESENCE IN SCHOOL WASTEWATER

Rebecca Fielding-Miller, Tommi Gaines, Ashkan Hassanl, Vinton Omaleki, Marlene Flores, Araz Majnoonian, Carrissa Wijaya, Richard Garfein

University of California San Diego, San Diego, CA, USA

Background: The SARS-CoV-2 virus is airborne and highly transmissible. Masking is an important strategy for source control and personal protection. The American Academy of Pediatrics recommends masking as part of a comprehensive strategy to reduce the spread of COVID-19 and respiratory diseases in school settings, however the effectiveness of school masking policies has been heavily debated. Previous studies of masking effectiveness have been limited by the use of self-reported masking behavior, policies as a proxy for masking behaviors, and/or case surveillance data that are biased by access to testing.

Methods: The Safer at School Early Alert (SASEA) project provided daily wastewater SARS-CoV-2 surveillance for elementary schools serving historically marginalized communities in San Diego County. We previously found that daily wastewater surveillance can identify 95% of PCR-detectable COVID-19 cases on campus. Between March 2 and May 27, 2022, we randomly selected 10 schools from the SASEA project for bi-weekly systematic observations of masking behaviors of students, staff, and parents. Each school was observed by 4 trained observers from the time school let out until all individuals had left. Observers counted the total number of adults and children and whether they were fully masked (nose and mouth covered), partially masked, or unmasked. We built a logistic regression model to measure the association between positive wastewater signals and the percentage of individuals who were observed fully masked vs partially or unmasked (primary predictor).

Results: We conducted 60 observation events over 6 weeks, during which positive wastewater signals—suggesting the presence of at least one COVID-19 case on campus—occurred on 9 days. On average, 38.6% of individuals were observed fully masked. After adjusting for intra-site correlation, observation week, current case rate per 100,000 in the school ZIP code and vaccination rate in the school ZIP code, we found that the odds of a positive wastewater signal in the 5 days after observation decreased by 47% (aOR 0.53, 95% CI: 0.28 – 0.99) for each 10% increase in the proportion of fully masked individuals.

Conclusion: Masking is an effective strategy to prevent the spread of COVID-19 in school settings. Even a relatively small increase in the proportion of individuals masking can potentially lead to a significant difference in epidemic spread.

211 VARIABLE REDUCTIONS IN SARS-CoV-2 VARIANT IMPORTATIONS FOLLOWING TRAVEL RESTRICTIONS

Angela McLaughlin, Jeffrey B Joy

British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada

Background: Retrospectively quantifying effectiveness of interventions such as travel restrictions to counter viral introduction and transmission is critical to inform public health policy. Phylogenetic analyses of SARS-CoV-2 variants were undertaken to quantify the effects Canadian COVID-19 travel restrictions had on variant importation and transmission dynamics.

Methods: Global and Canadian GISAID sequences available up to March 2022 were subsampled proportionally to variant-specific case counts and ten phylogenies were inferred for each variant. Trees, dates, and geographies were inferred using maximum likelihood.

Results: In response to Alpha, Canada implemented a UK flight ban from December 20, 2020—January 6, 2021, resulting in a 1.5-fold reduction in UK sublineage importation rate, with subsequent rebound (Fig. 1). Enhanced screening measures were implemented on December 24, 2020 for South African arrivals to counter Beta. Although there was a 6.3-fold reduction of Beta sublineages per week from South Africa following enhanced screening, there is low confidence in rare events. For Gamma, enhanced screening for arrivals from Brazil was implemented March 31–April 13, 2021. Proportion of Gamma sublineages from Brazil was reduced 1.6-fold within 2 weeks of the intervention, but the weekly importation rate was not significantly changed from start to end of intervention. In response to Delta, Canada issued a suspension of flights from India from April 22–September 23, 202, coinciding with a 2.4-fold reduction in sublineage importation and 3.8-fold reduction in proportion of sublineages from India. Increased importations from the USA and Europe progressively negated the ban’s effectiveness. Against Omicron, Canada banned entry of all foreign nationals who had travelled through southern Africa and implemented enhanced screening for Canadians from November 26—December 18, 2021. Subsequently, the BA.1 sublineage importation rate from South Africa was maintained at a low level amid rising cases, while importations from other sources increased, reducing the proportion of sublineages from South Africa and diluting the measure’s effectiveness.

Conclusion: Flight bans and enhanced screening against SARS-CoV-2 variants were most effective when implemented rapidly and for longer time; however, effectiveness declined as variants became globally widespread. Ongoing genomic surveillance programs incorporating phylogenetic analyses can inform travel restriction and non-pharmaceutical intervention policy.

Figure 1. Reduction of variant of concern (VOC) introductions from source nations following Canadian COVID-19 travel restrictions. A) The timing of travel restrictions in Canada against VOC. B) Fold reduction in sublineage importation rates and C) proportion of sublineages from source nations 2 weeks after restriction implementation. Error bars depict t-distribution 95% confidence intervals across ten bootstraps.
didactic attention-matched control condition in increasing COVID-19 testing uptake and acceptance of vaccination referrals. Based on Social Cognitive Theory, trained, SSP-hired peer counselors delivered tailored education, motivational interviewing, and problem-solving and planning to the active LinkUP intervention arm. We referred eligible participants (PWID, ≥18 years old, San Diego County residents without recent voluntary COVID-19 testing or fully vaccinated status) to mobile SSP sites that had been randomized by week to offer LinkUP as the control condition; all participants were then offered on-site rapid COVID-19 antigen testing and vaccination referrals. Our intent-to-treat analysis used Chi-square tests to compare intervention groups’ outcomes and log-bimomial regression to estimate preliminary intervention efficacy and explore potential moderation.

**Results:** Among 150 participants, median age was 41 years, 33% identified as Latinx and 65% as male, 73% were experiencing homelessness, and 45% had prior mandatory COVID-19 testing. Overall, we only detected one SARS-CoV-2 case. However, more active intervention vs. control participants agreed to COVID-19 testing (77.3% vs. 22.7%; p < .001) and vaccine referrals (32.4% vs. 13.3%; p = .006). Homelessness moderated intervention effects: LinkUP increased COVID-19 testing uptake more among participants experiencing homelessness (adjusted risk ratio [aRR]: 1.64; 95% CI: 1.27-2.12) than those not experiencing homelessness (aRR: 1.25; 95% CI: 0.99-1.56).

**Conclusion:** Findings from this RCT support the efficacy of LinkUP in increasing COVID-19 testing and acceptance of vaccination referrals among PWID presenting at mobile SSP sites, particularly for those experiencing homelessness. This research underscores the significance of community-academic partnerships when working with PWID and identifies a promising model that could be adapted to increase access to other underutilized vaccines in this vulnerable population.

### 213 CLUSTER-RANDOMIZED TRIAL COMPARING TWO SARS-CoV-2 TESTING MODELS IN AFRICA

**Nilesh Bhatt,1 Aida Yemaneeberhan,2 Boris K. Tchounga3, Leonie Simo4, Dijkeussi K. Taitiana,5 James Ndimbiri,5 Stephen Samba,6 Magoma Kivasta,6 Emiliene Epeve6, Anne Cecile Zones,7 Kanyi Bisiseki, Sharee Pearson,8 Rhoderick Machekane,9 Appolinaire Tiam10, Laura Guay11, Rose Macaba12,13**

1Elizabeth Glaser Pediatric AIDS Foundation, Washington, DC, USA; 2Elizabeth Glaser Pediatric AIDS Foundation, Yaoundé, Cameroon; 3Elizabeth Glaser Pediatric AIDS Foundation Cameroon, Yaoundé, Cameroon; 4Elizabeth Glaser Pediatric AIDS Foundation, Nairobi, Kenya; 5Ministry of Health Cameroon, Yaoundé, Cameroon; 6Elizabeth Glaser Pediatric AIDS Foundation Foundation, Washington, WA, USA; 7University of Nebraska Medical Center, Omaha, NE, USA; 8Virginia Commonwealth University, Richmond, VA, USA; 9University of Wisconsin—Madison, Madison, WI, USA; 10Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; 11Duke University, Durham, NC, USA; 12George Washington University, Falls Church, VA, USA

**Background:** Most programs use a screen and test strategy to identify SARS-CoV-2 infection, but this strategy does not identify individuals with asymptomatic infection. We determined the SARS-CoV-2 case detection rates in a test-all model compared to the standard screen-and-test model in Kenya and Cameroon.

**Methods:** A cluster-randomized trial was conducted in 20 health facilities between May-October 2022. In each country, 5 facilities were randomized to test all (testing offered regardless of screening outcome) or screen and test (testing offered if screened positive) arms. Additional staff were hired to support implementation of the two models in Kenya (K) and the test all model in Cameroon (C). Clients age >2 years attending HIV, TB and MNCH clinics were tested using SARS-CoV-2 rapid antigen tests. We estimated case detection rates (CDR) with facility level weighted averages and used a weighted t-test with robust standard errors for between arm comparison.

**Results:** Overall, 80,828 attendee visits were reported in the test-all arm (63,492 C and 17,336 K) and 71,254 attendee visits were reported in the screen-and-test arm (56,589 C and 14,665 K). In the test-all arm, 42,325 (52.4%) were screened for COVID-19 symptoms (46.7% C and 73.2% K) and 21,356 (26.6%) were tested (29.2% C and 17.4% in Kenya) with a positivity rate of 1.4% (2.0% C and 1.1% K). In the screen-and-test arm, 48,314 (67.8%) were screened (72.8% C and 48.6% K), and 3,629 (7.5%) were eligible for testing (8.2% C and 3.7% K) – of those, 2,139 (58.9%) were tested (57.1% C and 82.4% K) with a positivity rate of 4.1% (3.4% C and 10% K). The estimated CDR was 3.59 (95% CI 1.55-5.64) per 1,000 attendee visits in the test-all arm and 1.46 (95% CI 0.60-2.32) per 1,000 attendee visits in the screen-and-test arm. Compared to the screen-and-test arm, the test-all arm had significantly higher COVID-19 CDR in MNCH clinics (3.57 vs 1.29, p = 0.034). There were no significant differences in COVID-19 CDR between the two arms in HIV (4.20 vs 1.98, p = 0.174) and TB (10.33 vs 5.03, p = 0.283) clinics, though the number of SARS-CoV-2 infections was small.

**Conclusion:** The test-all arm identified more SARS-CoV-2 cases than the routine screen-and-test model, despite overall low testing coverage. The test-all model should be considered in future epidemics to improve early detection of SARS-CoV-2 infection among vulnerable populations, but effective implementation requires additional human resources to manage the clinic volumes.

### 214 COVID-19 BIVALENT BOOSTER EFFECTIVENESS IN PEOPLE WITH AND WITHOUT IMMUNE DYSFUNCTION

**Jing Sun1, Yifan Zhang1, Alfred J. Anzalone1, Amy L. Olex2, Eric Hurwitz3, Jomol Mathew4, Nasia Safdar5, Jessica Y. Ismael6, Roslyn Mannion7, Amanda J. Vinson8, Christopher Chute9, Melissa A. Haendel9, Rena Patel9, Greg Kirk2 National COVID Cohort Collaborative1**

1The Johns Hopkins University, Baltimore, MD, USA; 2The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 3University of Nebraska Medical Center, Omaha, NE, USA; 4Virginia Commonwealth University, Richmond, VA, USA; 5University of Wisconsin—Madison, Madison, WI, USA; 6University of Washington, Seattle, WA, USA; 7The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 8The University of Texas Southwestern Medical Center, Dallas, TX, USA; 9University of California, Davis, Davis, CA, USA; 10Merck & Co., Whitehouse Station, NJ, USA; 11University of California, San Francisco, San Francisco, CA, USA

**Background:** Data on the effectiveness of the bivalent booster vaccine against COVID-19 breakthrough infection and severe outcomes is limited.

**Methods:** Using patient-level data from 54 sites in the U.S. National COVID Cohort Collaborative (NCCC), we estimated bivalent booster effectiveness against breakthrough infection and outcomes between 09/01/2022 (bivalent vaccine approval date) to 12/15/2022 (most recent data release of N3C) among patients completed 2+ doses of mRNA vaccine. Bivalent booster effectiveness was evaluated among all patients and patients with and without immunosuppressed/compromised conditions (ISC; HIV infection, solid organ/bone marrow transplant, autoimmune diseases, and cancer). We used logistic regression models to compare the odds of breakthrough infection (COVID-19 diagnosis after the last dose of vaccine) and outcomes (hospitalization, ventilation/ECMO use, or death ≤28 days after infection) in the bivalent boosted vs. non-bivalent boosted groups. Models controlled for demographics, comorbidities, geographic region, prior SARS-CoV-2 infection, months since the last dose of non-bivalent vaccine, and prior non-bivalent booster.

**Results:** By 12/15/2022, 2,414,904 patients had received 2+ doses of mRNA vaccination, 75,873 of them had received a bivalent booster vaccine, and 24,046 of them had a breakthrough infection. At baseline, the median age was 52 (IQR 36-67) years, 40% male, 63% white, 10% Black, 12% Latinx, 3.5% Asian American/Pacific Islander, and 14% were patients with ISC. Patients received a bivalent booster were more likely to be female and had comorbidities. Bivalent booster was significantly associated with reduced odds of breakthrough infection and hospitalization (Figure). The adjusted odds ratios comparing bivalent vs. non-bivalent group were 0.28 (95% CI 0.25, 0.32) for all patients and 0.33 (95% CI 0.26, 0.41) for patients with ISC. Compared to the non-bivalent group, the bivalent group had a lower incidence of COVID-19-related hospitalization (151 vs. 41 per 100,000 persons), invasive ventilation/ECMO use (7.5 vs. 1.3 per 100,000 persons), or death (11 vs. 1.3 per 100,000 persons).
in all patients during the study period; the incidence of severe outcomes after bivalent boosting was similar among patients with and without ISC.

**Conclusion:** A bivalent booster vaccine was highly effective against COVID-19 breakthrough infection and severe outcomes among patients received 2+ doses of mRNA vaccine and offered similar protection in patients with and without ISC.

Figure. COVID-19 bivalent booster vaccine effectiveness against breakthrough infection and COVID-19 related hospitalization by patients with and without immune dysfunction in National COVID Cohort Collaborative.
**POSTER ABSTRACTS**

215 **LENACAPAVIR DISRUPTS HIV-1 CORE INTEGRITY WHILE STABILIZING THE CAPSID LATTICE**
Chenglei Li, Ryan C. Burdick, Wei-Shau Hu, Vinay K. Pathak
National Cancer Institute, Frederick, MD, USA

*Background:* Lenacapavir (LEN) is a potent HIV-1 capsid inhibitor (Gilead Sciences) currently in phase 2/3 clinical trials. Structural studies indicate that LEN binds two neighboring capsid subunits and stabilizes the cone-shaped capsid lattice, but its mechanism of viral inhibition and effect on capsids remain elusive. We recently utilized two different capsid-labeling approaches and demonstrated that intact HIV-1 capsids enter the nucleus and retain their integrity until just minutes before capsid disassembly (uncoating). Here, we sought to determine the effects of LEN on viral core integrity and kinetics of uncoating using isolated viral cores and cell-based assays.

*Methods:* We used two capsid-labeling methods: green fluorescent protein (GFP) fused to capsid (GFP-CA) and GFP as a fluid phase content marker (iGFP) by inserting GFP between matrix and capsid (GagGFP) that was proteolytically cleaved from Gag during virion maturation. Some of the GFP remains trapped inside capsids and serves as a reporter of capsid integrity loss. HIV-1 viral cores isolated through sucrose gradient fractionation were characterized by immunostaining and single virion analysis (SVA). SVA and live-cell imaging were then used to investigate the effects of LEN on isolated capsids and nuclear capsids in infected HeLa cells, respectively.

*Results:* Immunostaining showed that only capsids labeled with GFP-CA, not iGFP, were stained with anti-GFP antibody. In addition, GFP-CA, but not iGFP, inhibited Mx2 restriction of HIV-1 infection, indicating that GFP-CA is incorporated into the capsid lattice and exposed on the outside. The number of GFP-CA-labeled capsids either increased or remained constant upon LEN treatment, while the number of iGFP-labeled capsids was significantly reduced in a dose-dependent manner. Live-cell imaging showed that upon LEN treatment, GFP-CA-labeled nuclear capsids remained detectable for >1 hour, while iGFP-labeled nuclear capsids disappeared within 5 minutes.

*Conclusion:* Our results show that GFP-CA is an HIV-1 capsid lattice marker and iGFP is a reporter of core integrity. LEN treatment stabilizes the capsid lattice while disrupting core integrity. These results highlight the importance of retaining an intact capsid until shortly before integration. The high potency of LEN inhibition of HIV-1 replication may be explained in part by the disruption of core integrity, rather than inhibition of capsid disassembly.

216 **SINGLE-VIRUS IMAGING OF HIV-1 NUCLEAR IMPORT**
Anayat Bhat, Ivan Nombela, Barbara V. Remoortel, Frauke Christ, Zeger Debyser
Molecular Virology and Gene Therapy, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Katholieke Universiteit Leuven, Leuven, Belgium

*Background:* In search of a cure for HIV infection, understanding of all molecular determinants resulting in HIV persistence is warranted. The mechanism of the early steps of HIV-1 replication including capsid uncoating, nuclear import, and integration remain poorly understood. In particular, the intracellular localization and timing of capsid uncoating is a matter of debate. Evidence is emerging that nuclear import and integration sites determine the transcriptional state of the provirus. Viral infection is essentially heterogeneous in nature as only a few particles yield an integrated provirus. Conventional molecular biology techniques provide a limited understanding of the process at the ensemble level. Advanced microscopy methods offer the advantage to study the mechanism of viral replication at single virus level.

*Methods:* Our group has established an imaging platform to study viral infection at the single-particle level. We use fluorescently labelled integrase and time-lapse imaging to analyze the different steps of the replication cycle of HIV-1 in living cells. We follow the trajectory of the individual particles and their change in fluorescence intensity during their transport to the nucleus. We have also optimized super-resolution microscopy, STED (stimulated emission depletion) to estimate and compare the size of cytoplasmatic and nuclear preintegration complexes (PICs) with a high spatial resolution. To study the role of host factors TRN-SR2, TNPO1, and CPSF6 in nuclear import we generated cell lines depleted for each of these factors.

*Results:* We observe a 2-fold decrease in the fluorescence intensity signal and a 1.5-fold decrease in the size of nuclear PICs by confocal and STED imaging. Moreover, the nuclear import takes place after transient docking of one to two minutes of the viral particles at the nuclear membrane. In TNPO1, TRN-SR2, and CPSF6-depleted cell lines a 2 to 3-fold increase in the docking time, the time spent in the cytoplasm, and the overall import time of PICs were observed confirming their role in nuclear import.

*Conclusion:* A decrease in the fluorescence intensity and size of nuclear PICs corroborates that uncoating mainly takes place at the nuclear membrane. The depletion of TRN-SR2 hampered single-round infection by 60% whereas, the decrease in single-round infection in cell lines depleted for TNPO1 or CPSF6 was not significant pointing to a possible redundancy for the use of host factors in the process of nuclear import.

217 **RESTRICTION OF LENTIVIRAL CAPSIDS BY PRIMATE TRIM34**
Joy Twentyman, Abby Felton, Michael Emerman, Molly Ohanline
Fred Hutchinson Cancer Research Center, Seattle, WA, USA, University of Washington, Seattle, WA, USA, University of California Berkeley, Berkeley, CA, USA

*Background:* Human immunodeficiency virus (HIV) and other lentiviruses evolved to evade restriction factors, a type of germine-encoded, host innate immune proteins that inhibit viral replication. Understanding how restriction factors constrain lentivirus replication and transmission is key to understanding the emergence of pandemic viruses like HIV-1. The restriction factor TRIM5α can both mediate HIV capsid (CA) sensing and subsequent innate immune signaling and directly inhibit viral replication. Specifically, TRIM5α-mediated ubiquitination has been shown to induce downstream immune activation, while TRIM5α blocks replication by multimerizing onto the HIV core, inducing aberrant capsid uncoating. TRIM34, a paralog of TRIM5α, is a restriction factor of certain HIV and SIV capsids. Notably, TRIM34-mediated restriction requires TRIM5α, and TRIM5α-mediated restriction requires multimerization of TRIM5α monomers. Thus, we propose that TRIM34 requires multimerization with TRIM5α to restrict lentiviral capsids. The goals of this work are to identify determinants of antiviral specificity for TRIM34-mediated restriction, to identify domains of TRIM34 that are required for lentiviral restriction, and to examine TRIM34 and TRIM5α interactions.

*Methods:* To study the roles of TRIM5α and TRIM34 in restricting divergent lentiviruses, we co-expressed primate orthologues of TRIM5α and TRIM34 to test their ability to restrict a variety of lentivirus capsids. We generated chimeric and mutant TRIM proteins to identify regions of TRIM34 and TRIM5α that are necessary or sufficient for TRIM34-mediated restriction. To address whether TRIM34 is involved in signaling, we immunoprecipitated TRIM34 and TRIM5α to assess their capacity for ubiquitination.

*Results:* We found that diverse primate TRIM5α orthologues can restrict SIVagm and SIVmac capsids. This restrictive capacity appears broadly conserved across primates: all primate TRIM34 orthologues that we tested, regardless of species of origin, were able to restrict in the presence of human TRIM5α. Furthermore, we found that TRIM5α is necessary, but not sufficient, for restriction of these capsids. Finally, we found that TRIM34 is not polyubiquitinated to the same extent that TRIM5α is.

*Conclusion:* These data suggest that TRIM34 is a conserved primate lentiviral restriction factor. These studies will elucidate how TRIM34 and TRIM5α interact with each other and capsids, leading to a better understanding of TRIM34’s role in host-pathogen evolutionary history.

218 **HIV INFECTION OF Tfh: INTERROGATING THE SAMHD1 CONTRIBUTION THROUGH HIV-2**
André Pires, Rita Moura, Guilherme B. Farias, Carolina M. Conceição, Ana V. Antão, Tiago Ferreira, Bárbara Tavares, Amelia C. Trombeta, Ana Godinho-Santos, Ana E. Sousa
Instituto de Medicina Molecular, Lisboa, Portugal

*Presented at CROI by a nonauthor colleague*

*Background:* SAMHD1 is a host restriction factor with recognized impact in HIV reservoir establishment and suggested to be involved in follicular helper T cell (Tfh) biology. We explore here the ability of HIV-2 to counteract SAMHD1 due to the expression of Vpx, to further investigate the interplay of SAMHD1 and HIV in Tfh.

*Methods:* We sort-purified two distinct subsets of CD4+ T-cells from tonsillar tissue (n=6) known to feature distinct levels of SAMHD1, namely: Tfh, defined...
Neuf from primary HIV infection counteracts IFITM3 and restores virion infectivity

Mahesh Agarwal, Salila Majdoul, Abigail A. Jolley, Alex A. Compton

Background: Interferon-induced transmembrane protein 3 (IFITM3) is a restriction factor that reduces HIV-1 infectivity by incorporating into virions, inhibiting Env function, and reducing virion entry into cells. We previously found that the accessory protein of murine leukemia virus, glycopag enables viral evasion of IFITM3. However, whether other retroviruses encode the means for IFITM3 counteraction was unclear. Here, we investigated whether HIV-1 Nef reduces the impact of IFITM3 on virion infectivity.

Methods: Nef-deficient HIV-1 (NL4.3) was produced by transient transfection of HEK293T cells. Nef status was verified by PCR and flow cytometry. Cells were infected with Nef-deficient or wild-type HIV-1 and co-immunoprecipitation and western blot analysis was performed. The impact of Nef on IFITM3 subcellular localization was determined by immunofluorescence microscopy of cells transfected with EEA1-GFP.

Results: Expression of IFITM3 in cells producing Nef-deficient NL4.3 HIV resulted in a 4- to 6-fold loss of virion infectivity. Using Nef from HIV-1 isolated from transfected/founder to lab-adapted molecular clones, we found that certain Nef variants exhibit the capacity to restore virion infectivity in the presence of IFITM3. Nef derived from primary infection (97ZAG12, Clade C) fully restored HIV-1 infectivity in presence of IFITM3, while the effect of Nef on NL4.3 was modest. Mutagenesis of Nef revealed that membrane anchoring and interactions with endocytic machinery were critical for overcoming IFITM3. Furthermore, Nef from at least some transfected/founder strains (including CH040 and SUMA) also conferred resistance to IFITM3. Mechanistically, the relative ability of Nef variants to co-immunoprecipitate IFITM3 was associated with their ability to restore virion infectivity. Furthermore, co-expression of Nef and IFITM3 resulted in the enrichment of IFITM3 in early endosome and a reduction of IFITM3 at the cell surface.

Conclusion: Our results reveal a previously unrecognized activity of Nef that involves contributions from other Simian immunodeficiency viruses.

Vpu of an SIV isolate can target human BST-2

Weitong Yao, Akhil Chennuru, Preston Moore, Klaus Strebel, Tetsuro Matano, Taisuke Izumi, Takeshi Yoshida

Background: HIV-1 Vpu enhances the release of viral particles from infected cells by interfering with host factor BST-2/tetherin, which tethers nascent virions to the cell surface. The activity of Vpu is species-specific. Indeed, Vpu derived from HIV-1 but not SIV could target human BST-2, whereas some SIV Vpus but not HIV-1 Vpu could counteract monkey BST-2. HIV-1 is believed to have derived by zoonosis of SIVcpz. However, since Vpu encoded by SIVcpz neither antagonizes chimpanzee BST-2 nor human BST-2, HIV-1 Vpu was thought to have acquired the activity after virus transmission to humans. Interestingly, Vpu of SIVgsn71-99CM71 (SIVgsn71) isolated from Greater Spot-Nosed monkey (Ceropithecus nitidus, GSN monkey) counteracted human as well as GSN BST-2. The purpose of our current research therefore was to gain a more in depth understanding of how SIVgsn71 Vpu antagonizes human BST-2 and whether it could indeed be the prototype of HIV-1 Vpu.

Methods: To identify amino acids in SIVgsn71 Vpu critical for downregulation of human BST-2, cells expressing endogenous human BST-2 were infected with HIV-1 carrying WT or mutants of the SIV vpu gene. The downregulation efficiency of BST-2 was assessed by flow cytometry. We also performed a gain-of-function study using SIV Vpus normally inactive against human BST-2 (e.g., Vpu derived from SIVgsn166) to assess whether these Vpus can acquire activity against human BST-2 through targeted mutagenesis.

Results: We identified L21, A22, A25, A29, W30, K32, and W33 as important amino acids for SIVgsn71 Vpu to counteract human BST-2. The AxxxAAAxx motif in SIVgsn71 Vpu (A22, A25, and W33) was conserved in HIV-1 Vpu (A14, A18, and W22), but the other identified amino acids (L21, A22, W30, and K32) were unique to SIVgsn71 Vpu. Interestingly, a single amino acid replacement in SIVgsn166 Vpu was sufficient to render it active against human BST-2.

Conclusion: We found that Vpu of the SIVgsn71 isolate can target BST-2 from humans as well as from its natural host. In contrast, Vpu from a related virus isolate, SIVgsn166, was unable to target human BST-2. However, a single mutation in the SIVgsn166 Vpu gene rendered it active for human BST-2. Our results suggest that the origin of HIV-1 may be more complex than thought and involve contributions from other Simian immunodeficiency viruses.

Stinging CD4+ T cells to render them refractory to HIV infection

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Background: HIV rapidly infects CD4+ T cells where it can establish latent infection. Identifying immune mechanisms which can render CD4+ T cells refractory to infection represents a critical step in limiting infection and viral spread during acute infection. In this study, we identify that the STING signaling pathway functions in CD4+ T cells and can be activated to mediate a cellular state refractory to HIV infection.

Methods: Using purified memory CD4+ T cells from healthy human donors, we stimulated cells with titrating doses (1.0 mM - 50 mM) of the STING agonist diABZI for 18 hours followed by infection with HIV 89.6. p24 levels were quantified by flow cytometry (FCM) after 72 hours of infection. We next identified the critical role for STING signaling in HIV of limiting infection by CRISPR mediated knockdown (KD) of STING in purified CD4+ T cells. Control (CTL) and STING KD cells were infected and p24 quantified, as described before. Finally, purified memory CD4+ T cells were treated with 1 mM diABZI for 24 hours and global proteomics performed by Mass Spectrometry to identify the protein signatures induced and suppressed by STING activation.

Results: We found that 0.5 mM diABZI was sufficient to induce a state of refractoriness to HIV infection equivalent to IFN-α2a treatment in central memory CD4+ T cells, a major source of the HIV reservoir (Fig. 1A). We also have shown that diABZI induces Type I IFN production from CD4+ T cells and activates p-IRF3. CRISPR KD of STING in memory CD4+ T cells was shown to significantly enhance the level of HIV infected cells in vitro. Proteomics analysis revealed that HIV restriction factors and proteins involved in antiviral immunity and IFN signaling were significantly induced by diABZI. Importantly, proteins that were significantly reduced mapped to pathways including RNA processing and

as CXCR5negICOSnegPD-1neg, using FACS Aria (BD). Both Tfh and TN subsets were established of Tfh reservoirs, and their implications for the benign course of HIV-2 primary isolates prompt the investigation of additional paths in the advantage to infect tonsillar CD4 T cells, irrespectively of their SAMHD1 levels, and HIV-1 primary isolates. Surprisingly, the frequency of the generated Tfh and Tfh differentiation from circulating naïve CD4 T cells. Of note, the levels of cell-expression of SAMHD1 in TN, the levels of both proviral DNA and gag mRNA were compared to TN upon 24h for all the viral isolates. Nevertheless, despite the high expression of SAMHD1 in TN, the levels of both proviral DNA and gag mRNA were comparable upon HIV-2 and HIV-1 infections. Contrarily to HIV-1, CXCR4 usage in the case of HIV-2 was not associated with higher proviral DNA in both subsets, suggesting a minor impact of the type of co-receptor. Then, we asked whether HIV-2 infection biased Tfh generation using a standardized protocol for in-vitro Tfh differentiation from circulating naïve CD4 T cells. Of note, the levels of cell-associated proviral DNA were similar 72h post-infection with dual-tropic HIV-2 and HIV-1 primary isolates. Surprisingly, the frequency of the generated Tfh and their detailed phenotype were remarkably similar in all conditions.

Conclusion: HIV-2, despite harboring Vpu, did not feature a significant advantage to infect tonsillar CD4 T cells, irrespectively of their SAMHD1 levels, as well as to modulate in-vitro Tfh differentiation. These first data using HIV-2 primary isolates prompt the investigation of additional paths in the establishment of Tfh reservoirs, and their implications for the benign course of HIV-2.
**Conclusion:** Our data provide mechanistic insight into the STING pathway in CD4+ T cells as a means to mediate refractoriness to HIV infection and shows this pathway lands a “1-2” punch of heightening antiviral immunity while suppressing host machinery needed for viral replication. The prospect of treating with a STING agonist as part of post-exposure therapies represents a novel and impactful means of helping stop HIV infection in its tracks while restoring the normal antiviral immune response need to control the remaining virus.

Pathway analysis of proteins induced and reduced by diABZI treatment of memory CD4+ T cells

**222 PAN-CASPASE INHIBITION PREVENTS HIV REPLICATION BY THE INDUCTION OF THE IFN I PATHWAY**

Jordi Senzerrich, Sonia Pedroche, Elisabet Garcia, Elisabet Gómez, Bonaventura Clotet, Cecilia Cabrera

**Background:** Viral infection can trigger apoptosis and pyroptosis, which may limit viral replication. However, cleavage of critical molecules of the innate immune signaling pathways by activated caspases may also be of benefit for the virus. We hypothesize that inhibition of caspase activity by the pan-caspase inhibitor Q-VD-OPh can prevent HIV infection by the upregulation of the IFN I antiviral pathway.

**Methods:** We have determined the effects of Q-VD-OPh on HIV infection in a human tonsil ex vivo culture system. Caspase activation, cell death and viral replication have been analyzed in HIV-infected tonsil cells. Expression of activation markers, IFN and IFN-stimulated genes, as well as mitochondrial status have been assessed in uninfected tonsil cells.

**Results:** Q-VD-OPh prevented HIV-induced CD4+ T cell depletion and severely inhibited viral replication, decreasing the amount of viral p24 and total and integrated DNA in CD4+ T cells. This inhibitory effect was shown to be envelope-independent, as Q-VD-OPh could block infection by VSV-pseudotyped HIV viruses. Additionally, the mechanism of action was found to be caspase-dependent, since the effect was not observed with Q-VE-OPh, which lacks caspase inhibitory activity. Remarkably, HIV infection was still inhibited even when the compound was removed at the time of infection, suggesting Q-VD-OPh induces intrinsic cellular changes that render cells less susceptible to infection. Surprisingly, when evaluating the effect of Q-VD-OPh on cells in the absence of infection, Q-VD-OPh treatment resulted in the modulation of the IFN type I pathway, leading to increased expression of IFNβ and other IFN-stimulated genes, both in CD3+ and CD3- cells. This type IFN induction was responsible for the inhibition of HIV replication, as a cocktail of neutralizing anti-IFN and anti-IFN receptor antibodies could abrogate Q-VD-OPh restriction. Mechanistically, the activation of the IFN pathway by Q-VD-OPh was linked to changes in mitochondrial membrane potential, as this compound triggered a sublethal mitochondrial depolarization, which did not involve cell death or mitochondrial structure defects.

**Conclusion:** We propose that Q-VD-OPh induces sublethal mitochondrial depolarization, leading to the release of mitochondrial DNA into the cytosol. ThIs mtDNA can then be detected by cGAS/STING, resulting in the upregulation of type I IFN pathway genes. In summary, Q-VD-OPh inhibits HIV infection by a novel mechanism of action, driving cells into a potent anti-viral state.

**223 THE SPIKE OF SARS-CoV-2 VARIANTS ALLOWS FOR MORE EFFICIENT COUNTERACTION OF BST2**

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**Background:** BST2/Tetherin is an interferon-stimulated gene with antiviral activity against enveloped viruses. Particularly, BST2 tethers virions at their site of assembly, preventing their release and spread. In addition to this primary role, BST2 is as an important bridge between the innate and adaptive immune system, since (i) BST2 routes tethered particles to lysosomes, which generates viral breakdown products that engage pattern recognition receptors; and (ii) trapped virions facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). In turn, viruses have evolved mechanisms to bypass BST2, either by targeting BST2 for proteosomal/lysosomal degradation or by removing BST2 from sites of virion assembly. However, the role of BST2 in SARS-CoV-2 replication, spread, evolution, and pathogenesis remains largely unknown.

**Methods:** The antiviral potential of BST2 against SARS-CoV-2 was investigated by infecting different SARS-CoV-2 isolates (Hong Kong, Alpha, Beta, Delta, and Omicron) in BST2+ and BST2- cells. Culture supernatants were collected to assess virion production by ELISA and infectivity by TCID50. Infected cells were analyzed by western blot and flow cytometry to examine viral and cellular protein levels, including BST2. Transfection of individual SARS-CoV-2 ORFs and mutagenesis studies allowed us to identify the genes that the virus uses to downregulate BST2. Immunoprecipitation assays revealed protein–protein interactions and changes in ubiquitin patterns. Experiments with proteasomal and lysosomal inhibitors furthered our mechanistic understanding of how SARS-CoV-2 counteracts BST2. Finally, fluorescent microscopy studies uncovered changes in the subcellular distribution of BST2 in SARS-CoV-2 infected cells.

**Results:** While BST2 reduces virion release, SARS-CoV-2 has evolved to counteract this effect. Specifically, SARS-CoV-2 uses the Spike to interact with BST2, sequester the protein at perinuclear locations, and ultimately route it for lysosomal degradation. By surveying different SARS-CoV-2 variants of concern (Alpha-Omicron), we found that each variant is more efficient than the previously circulating strain at downregulating BST2 and facilitating virion production, and that mutations in the Spike account for their enhanced BST2 antagonism.

**Conclusion:** As part of its adaptation to humans, SARS-CoV-2 is improving its capacity to counteract BST2, highlighting that BST2 antagonism is important for SARS-CoV-2 infectivity and transmission.

**224 cGAS-STING PATHWAY LIMITS SARS-CoV-2 REPLICATION IN ACE2+ AIRWAY CELL LINES**

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**Background:** We previously screened 10 human lung and upper airway cell lines expressing variable levels of endogenous ACE2/TMPRSS2. We found that HS22 human lung adenocarcinoma cells supported SARS-CoV-2 replication independent of ACE2, whereas the ACE2 positive cell lines were not permissive to infection. Type I/III interferons (IFNs) potently restrict SARS-CoV-2 replication through the actions of hundreds of interferon-stimulated genes (ISGs) that are upregulated upon IFN signaling. Here we report that a number of ACE2 positive airway cell lines are unable to support SARS-CoV-2 replication due to basal activation of the cGAS-STING DNA sensing pathway and subsequent upregulation of IFNs and ISGs which restrict SARS-CoV-2 replication.

**Methods:** SARS-CoV-2 WT strain 2019-nCoV USA-WA1/2020 viral replication was detected through analysis of cell associated RNA. RNA sequencing was used to study the basal level of genes in the type-I IFN pathway in the 10 cell lines, which was further validated by western blotting and qRT-PCR. A panel of 5 cell lines, with varying expression levels of ACE2 and TMPRSS2, were pre-treated with Ruxolitinib, a JAK1/2 inhibitor. A siRNA-mediated screen was used to determine the molecular basis of basally high expression of ISGs in cell lines. CRISPR knockout of IFN-alpha receptor and cGAS-STING pathway components was conducted in parallel.

**Results:** Here we show that higher basal levels of IFN pathway activity underlie the inability of ACE2+ cell lines to support virus replication. Importantly, this IFN-induced block can be overcome by chemical inhibition and genetic disruption of the IFN signaling pathway or by ACE2 overexpression, suggesting...
that one or more saturable ISGs underlie the lack of permissivity of these cells. Ruxolitinib treatment increased SARS-CoV-2 RNA levels by nearly 3 logs in OE21 and SCC25. Furthermore, the baseline activation of the STING-cGAS pathway accounts for the high ISG levels and genetic disruption of the cGAS-STING pathway enhances levels by nearly 2 and 3 logs of virus replication in the two separate ACE2+ cell line models respectively.

**Conclusion:** Our findings demonstrate that cGAS-STING-dependent activation of IFN-mediated innate immunity underlies the inability of ACE2+ airway cells to support SARS-CoV-2 replication. Our study highlights that in addition to ACE2, basal activation of cGAS-STING pathway, IFNs and ISGs may play a key role in defining SARS-CoV-2 cellular tropism and may explain the complex SARS-CoV-2 pathogenesis in vivo.

**225** **LONG NON-CODING RNA REGULATES IFN-I RESPONSE AND SARS-COV-2 REPLICATION**

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**Background:** Infection with SARS-CoV-2 triggers reprogramming through global transcriptional changes that drive the development of Coronavirus disease 2019 (COVID-19). Although the expression and functions of protein-coding transcripts have been widely studied in SARS-CoV-2 infection, most of the transcriptome consists of non-protein-coding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs), which constitute a large proportion of the transcriptome, regulate immune responses and play prominent roles in health and disease. However, the impact of lncRNAs on SARS-CoV-2 infection is poorly understood. Our study will provide fundamental insights into the role of lncRNAs in SARS-CoV-2 infection.

**Methods:** We hypothesized that SARS-CoV-2-induced lncRNAs are critical regulators of viral replication and immune response. To test our hypothesis, we identified lncRNAs with significant differential expression in SARS-CoV-2 infected vs. uninfected cells across two cell types (A549-hACE2 and Calu) from published transcriptome data. We silenced the expression of the top lncRNA Bre-AS1 (BA) a human lung epithelial cell model (A549 cells stably expressing HAEC2 and hTMPRSS2, AS1) using lncRNA-specific ASO (lncsi) or negative control AS1. Published transcriptome data show that cellular lncRNAs could play significant roles in immune response and viral propagation. Thus, unraveling the mechanisms of lncRNA-mediated regulation of virus replication and immune response may lead to identifying new, highly selective therapeutic targets.

**Bre-AS1 inhibits STAT4 phosphorylation and enhances ISG transcription**

**Results:** Our data show that cellular lncRNA, Bre-AS1 enhances antiviral interferon-stimulated genes (ISGs). Tyr705 pSTAT3 forms suppressor molecular complexes (pSTAT3-pSTAT1 or pSTAT3-PLS2CR) that inhibit ISG transcription. Using molecular methods such as gene-silencing, immunoprecipitation, western blot, and measuring promoter activity, we further show that Bre-AS1 inhibits the phosphorylation of STAT3 and enhances ISG transcription.

**Conclusion:** Cellular lncRNA Bre-AS1 enhances expression of antiviral interferon-stimulated genes (ISGs) expression and inhibits replication of SARS-CoV-2. Our data show that cellular lncRNAs could play significant roles in immune response and viral propagation. Thus, unraveling the mechanisms of lncRNA-mediated regulation of virus replication and immune response may lead to identifying new, highly selective therapeutic targets.

**227** **Vpr ENHANCES HIV REPLICATION BY INHIBITING T CELL PROLIFERATION**

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**Background:** The resting HIV reservoir is a major obstacle towards curing HIV+ people. The reservoir is composed of clonally expanded T cells that predominantly harbor defective proviruses. A few infected cells containing replication-competent provirus are responsible for rebound viremia after treatment interruption. It is known the Vpr protein inhibits proliferation in cell lines. However, the timing of Vpr expression in resting infected cells is unknown and whether it will prevent proliferation of cycling primary CD4 T cells with replication-competent HIV.

**Methods:** We explored this question by direct infection of primary resting CD4 T cells with a CCR5-tropic replication-competent GFP reporter virus. We measured GFP expression by flow cytometry over 5 days. We performed bulk and single cell RNAseq of sorted GFP+ cells to measure host and virus mRNAs. We measured proliferation by first labelling resting infected cells with violet CellTrace and then stimulating them for 10 days with anti-CD3/CD28 beads and ART. We infected cells with wild-type Vpr, without Vpr, and with Vpr mutants known to affect T cell proliferation. We measured cell cycle progression using the mitosis marker phospho-Ser31 in histone 3.3 in both resting and stimulated cells with ART. Finally, we assayed HIV replication through co-culture of sorted infected cells with autologous or heterologous target primary CD4 T cells.

**Results:** We detected resting GFP+ cells 3 to 4 days after infection. Transcriptome analysis of resting GFP+ revealed infected cells expressed mRNAs coding for Vif, Vpr, VpuEnv and Nef. We confirmed Vpr modulates progression through the cell cycle and prevents T cell proliferation. Infected cells lacking Vpr contained transcriptionally-silent proviruses in the divided cells. In contrast, transcriptionally-active proviruses were present in the divided cells that expressed non-functional Vpr, because of mutations that prevented its binding to the host ubiquitin chaperone protein DCAF1. Lastly, we found replication-competent HIV originated from the undivided cells instead of the divided cells.

**Conclusion:** We hypothesize that denser HIV populations may have higher rates of coinfection and therefore recombination. However, this potential impact of viral density on recombination has not been previously quantified due to a lack of methods that can sensitively estimate recombination without extensive genetic data.

**Vpr modulates progression through the cell cycle and prevents T cell proliferation.**

**Methods:** To bridge this gap, we develop a method to quantify recombination in genetic time series data based on the autocorrelation of linkage between SNPs across timepoints. We validate this method on extensive simulated data and confirm its accuracy at recovering recombination rates under widely used short read sequencing conditions.

**Results:** We apply our new method to longitudinal, high-throughput viral sequencing data from PLWHIV, stratifying populations by viral load (a proxy for density) to investigate whether density impacts recombination rate. In populations with viral loads below 5×10^4 copies/mL, we estimate a recombination rate of 2.4±10^−6 events/bp/generation (95% CI 1.5×10^−6–3.9×10^−5), similar to existing estimates. However, in populations with viral loads above 5×10^4 copies/mL, we estimate significantly higher rates of viral recombination (7.5±10^−6 events/bp/generation, 95% CI 5.5×10^−6–9.8×10^−5). This elevation of viral recombination rate in populations with high viral loads persists across a number of viral load thresholding choices. We find a similar association of viral load and recombination rate within a single individual with varying viral load over time.

**Conclusion:** Our findings suggest that recombination rates can vary across PLWHIV and within those people over time, deepening our understanding of this otherwise static parameter. Elevated recombination rate during periods of high viral load provides an additional hypothesized mechanism for how HIV diversifies during acute and/or uncontrolled infection and suggests that incorporating variation in recombination rate estimates could lead to new insights from intra-host genetic data.
A HUMAN-SPECIFIC CARD8 MUTATION AFFECTS INFLAMMASOME ACTIVATION AFTER HIV-1 INFECTION

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Background: Humans have a variety of innate immune sensors including inflammasomes which are cytosolic innate immune complexes that can sense and respond to pathogens. It was recently shown that the caspase recruitment domain-containing protein 8 (CARD8) inflammasome is activated by recognize the enzymatic activity of HIV-1 protease (HIV-1PR), resulting in a lytic form of cell death called pyroptosis and the release of proinflammatory cytokines when a nonnucleoside reverse transcriptase inhibitor (NNRTI) was used to enforce protease dimerization (Wang et al 2021). Here, we took an evolution-guided and virological approach to infer the significance of CARD8's interaction with different lentiviral proteases in the context of intact proviruses.

Methods: Among available primate CARD8 sequences, only human CARD8 has a phenylalanine at the P’ site of the HIV-1 protease cleavage site. We assessed the ability of HIV's and SIVcpz protease to cleave different CARD8 variants using an overexpression system. Additionally, we assessed whether or not CARD8 sensing and inflammasome activation occurs during HIV-1 infection using THP-1 cells that were knocked out for the CARD8 gene and then complemented back with different variants of CARD8.

Results: We demonstrated that human CARD8 has a unique motif among hominoids, Old World monkeys and gibbons that renders it susceptible to cleavage by HIV-1PR and SIVcpz protease, indicating that the precursor viruses to HIV-1 were poised to cleave human CARD8, but do not cleave chimpanzee CARD8. Further, we show that human CARD8, but not chimpanzee CARD8, can sense lentiviral protease activity and induce inflammasome activation in the context of HIV-1 infection in vitro, but only if the target cells are first transcriptionally primed with toll-like receptor (TLR) stimulation prior to viral challenge.

Conclusion: As a major hallmark of acute HIV-1 disease is circulating microbial ligands like lipopolysaccharides caused by the breakdown of the gut epithelial barrier, we hypothesize that CARD8-induced pyroptosis may be a major driver of HIV-1 pathogenesis contributing to depletion of the T cell population and chronic inflammation that characterize HIV/AIDS disease and may partially explain increased pathogenesis of HIV-1 in humans relative to SIVcpz in chimpanzees.

CARD8 INFLAMMASOME MODULATES HIV-1 INFECTION IN CD4+ T CELLS

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Background: CARD8 is an innate immune sensor that can be activated through proteolytic cleavage. Recently, we reported that induction of HIV-1 protease activation leads to CARD8 inflammasome-mediated pyroptosis of infected macrophages and CD4+ T cells. However, it remains unclear whether natural HIV-1 infection can activate the CARD8 inflammasome to trigger pyroptosis of CD4+ T cell and thereby modulating HIV-1 replication.

Methods: We co-cultured HIV-1-infected CD4+ T cells with CFSE-labeled control PBMCs (target cells) to assess the CD4+ T cell death and inflammasome activation. We also infected CD4+ T cells with replication-competent HIV-1 in vitro and in vivo to examine the role of CARD8 inflammasome in viral replication.

Results: We found that HIV-1 infection induces rapid CARD8-dependent pyroptosis of CD4+ T cells in peripheral blood, tonsil tissues, and humanized mice. Mechanistically, the N-terminus of CARD8 is cleaved by HIV-1 protease encapsulated in the incoming viral particles immediately after viral entry, which creates an unstable neo-N terminus, resulting in proteasome degradation of CARD8 and release of the bioactive C-terminal fragment for inflammasome assembly and pyroptosis. We also observed that deletion of CARD8 significantly reduced HIV-1-triggered cell death, leading to increased susceptibility to HIV-1 infection in CD4+ T cells. Furthermore, we showed that sensitization of CARD8 by DPP9 inhibitor suppressed HIV-1 infection in CD4+ T cells.

Conclusion: This study demonstrates that HIV-1 infection activates the CARD8 inflammasome in CD4+ T cells, which provides critical insights into how the CARD8 modulates HIV-1 infection and pathogenesis. This work elucidates sensitization of CARD8 by DPP9 inhibitor restricts HIV-1 infection, suggesting that targeting CARD8 inflammasome may facilitate the development of new therapeutics for HIV-1.

VHH DIMER P559-R45L NEUTRALIZES SARS-CoV-2 BY STABILIZING SPIKE IN UP CONFORMATION

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Background: SARS-CoV-2 Omicron subvariants are highly resistant to vaccine-induced immunity and therapeutic monoclonal antibodies. We previously reported anti-SARS-CoV-2 spike alpabodanobodies (VHHS) P86 and P17 that potently neutralize the wild type and VOCs from Alpha to Omicron BA.1 and BA.2, but not Omicron subvariants after that such as BA.4/5. Thus, we tried to establish a new VH that can neutralize all the variants including BA.4/5.

Methods: We developed VH1 trimers and heterodimers based on the structural and computational analysis of Delta spike-immunized alpabodan VH library. We tested representative VHHS against SARS-CoV-2 spike by pseudovirus assays and generated VH1 heterodimers. We further obtained Crypto-EM structure of Spike trimer and VH1 monomer or heterodimer.

Results: First, we generated series of P86 mutants to counteract L452R mutation in Delta or Omicron BA.1 subvariants and found that P86-R45L was most potent against D614G with an IC50 of 0.03 μg/mL. From the Delta spike-immunized VH library, we also identified that homo-trimer of a new clone P559 neutralized SARS-CoV-2 Delta and Omicron BA.5 variants with IC50 of 0.077 and 0.54 μg/mL, respectively. We finally generated P559-R45L heterodimer that neutralized all the variants so far including Omicron BA.5 with an IC50 of 0.39 μg/mL. Crypto-EM structure revealed that three molecules of P559-R45L heterodimer bridged two RBD molecules in the spike trimer and stabilized spike trimers with RBD in the up conformation.

Conclusion: We developed VH1 P559-R45L heterodimer that potently neutralized all the variants including Omicron subvariants through unique structural interaction.

VHH DIMER P559-R45L NEUTRALIZES SARS-CoV-2 BY STABILIZING SPIKE IN UP CONFORMATION

Kayoko Nagata1, Junso Fujita2, Ryota Maeda1, Kotaro Shirakawa1, Tsuyoshi Inoue3, Keichi Namba1, Akihito Imura1, Akifumi Takaori-Kondo1
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Background: SARS-CoV-2 Omicron subvariants are highly resistant to vaccine-induced immunity and therapeutic monoclonal antibodies. We previously reported anti-SARS-CoV-2 spike alpabodanobodies (VHHS) P86 and P17 that potently neutralize the wild type and VOCs from Alpha to Omicron BA.1 and BA.2, but not Omicron subvariants after that such as BA.4/5. Thus, we tried to establish a new VH that can neutralize all the variants including BA.4/5.

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Fc RECEPTOR-MEDIATED INFECTION OF MYELOID CELLS BY SARS-CoV-2

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Background: The role of myeloid cells in the pathogenesis of SARS-CoV-2 is well established, in particular as drivers of cytokine production and systemic inflammation characteristic of severe COVID-19. However, the potential for myeloid cells to act as bona fide targets of productive SARS-CoV-2 infection remains unclear.

Methods: Using anti-SARS-CoV-2 mAbs with a range of neutralisation potencies and binding specificities, we performed a detailed assessment of mAb-mediated infection of monocytes/macrophages. THP-1 cells were used as a model system, with results confirmed in primary macrophages.

Results: Infection of THP-1 cells was seen via mAbs targeting the spike RBD, but not with those targeting the NTD or S2 subunit, mAbs with the most consistent potential to mediate infection targeted a conserved region of the RBD (group 1/class IV). No infection was seen with the same quantity of virus but in the absence of antibody, and pre-treating the cells with FcγRI and -II blocking antibodies inhibited infection. Thus, antibody-FcR interactions are able to expand the tropism of SARS-CoV-2. Time-course studies demonstrated high-level and productive infection. Studies performed in human iPS-derived macrophages and primary monocyte-derived macrophages paralleled results seen in THP-1 cells but with lower infection levels. Up to 2% of macrophages were infected, with infected cells appearing multinucleated and syncytial. Addition of ruxolitinib, an inhibitor of JAK1/2 signalling, increased infection up to 10-fold, indicating limitation of infection through innate immune mechanisms. Sera from primary infections (n=80) mediated rare infection events, with a minority of samples (n=3) promoting significant infection. Competition assays confirmed results seen in sera, with the addition of neutralising mAbs diminishing the infection seen with infection-mediating mAbs. Thus, the presence of antibodies with potential to mediate infection is not sufficient to predict myeloid cell infection, rather, the context in which the antibodies are produced is key.

Conclusion: We hypothesise that a nascent antibody response during peak viral replication in primary infection presents a window of opportunity for myeloid cells to become infected, while establishment of a robust polyclonal response via vaccination or prior infection reduces the likelihood of this occurring.
Infection via antibody-FcR interactions could contribute to pathogenesis in primary infection, systemic virus spread or persistent infection.

232 OMICRON SPIKE ENDOWS SARS-CoV-2 WITH ENHANCED INFECTIVITY IN NASAL EPITHELIUM
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Background: Omicron lineages, including BA.1 and BA.2, emerged following mass COVID-19 vaccination campaigns, displaced previous SARS-CoV-2 variants of concern worldwide, and gave rise to sublineages that continue to spread among humans. Previous research has shown that Omicron lineages exhibit a decreased propensity for lower respiratory tract (lung) infection compared to ancestral SARS-CoV-2, which may explain the decreased pathogenicity associated with Omicron infections. Nonetheless, Omicron lineages exhibit an unprecedented transmissibility in humans, which until now has been solely attributed to escape from vaccine-induced neutralizing antibodies.

Methods: We comprehensively analyzed BA1 and BA2 infection in primary human nasal epithelial cells cultured at the air-liquid interface, which recapitulates the physiological architecture of the nasal epithelium in vivo. Meanwhile we also took advantage of the VSV-based pseudovirus decorated with different Spike variants.

Results: In primary human nasal epithelial cells cultured at the air-liquid interface, which recapitulates the physiological architecture of the nasal epithelium in vivo, BA.1 and BA.2 exhibited enhanced infectivity relative to ancestral SARS-CoV-2. Using VSV-based pseudovirus decorated with different Spike variants, we found that increased infectivity conferred by Omicron Spike is due to superior attachment and entry into nasal epithelial cells. In contrast to ancestral SARS-CoV-2, invasion of nasal epithelia by Omicron occurred via the cell surface and endosomal routes of entry and was accompanied by elevated induction of type-I interferons, indicative of a robust innate immune response. Furthermore, BA.1 was less sensitive to inhibition by the antiviral state elicited by type-I and type-III interferons, and this was recapitulated by pseudovirus bearing BA.1 and BA.2 Spike proteins.

Conclusion: Our results suggest that the constellation of Spike mutations unique to Omicron allow for increased adherence to nasal epithelia, flexible usage of virus entry pathways, and interferon resistance. These findings inform our understanding of how Omicron evolved elevated transmissibility between humans despite a decreased propensity to infect the lower respiratory tract. Additionally, the interferon insensitivity of the Omicron Spike-mediated entry process may explain why Omicron lineages lost the capacity to antagonize interferon pathways compared to ancestral SARS-CoV-2.

233 SARS-CoV-2 REPLICONS REVEAL SPIKE-INDEPENDENT ATTENUATION OF THE OMICRON VARIANT
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Background: The SARS-CoV-2 Omicron variant is highly immune evasive but is attenuated in cell and animal models of infection, which many reports attribute to spike mutations. However, the phenotype and contribution to viral fitness of Omicron non-spike mutations remain unknown.

Methods: To study mutations across the entire genome independent of spike, we developed a novel cloning and replicon system capable of generating mutants within 6 hours and obtaining phenotypic results within 3-4 days.

Results: Using a series of Omicron replicons, we found that ORF1ab harbors critical mutations, especially in the nonstructural protein 6 (NSP6), which lower viral fitness and are currently evolving in Omicron subvariants. In addition, Omicron mutations in several NSPs epistatically interact and are critical for viral replication and polyprotein processing.

Conclusion: Collectively, we describe a robust replicon technology to study mutations across the genome and our data highlight the need to vigilantly study and monitor non-spike mutations in emerging Omicron subvariants.

234 SARS-CoV-2 USES ENDOosomal MEMBRANES TO BUILD REPLICATION ORGANELLES
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Background: The health emergency caused by the COVID-19 pandemic has evidenced that the frequency of spillover episodes of viruses infecting bats to other species, including humans, has significantly increased compared to previous decades. Besides SARS-CoV-2, six other human coronaviruses (NL63, 229E, OC43, HKU1, SARS-CoV and MERS-CoV) emerged in the 20th and 21st century, most likely because of cross-species transmission events from bats. While many of these coronaviruses cause mild respiratory infections, MERS-CoV, SARS-CoV and SARS-CoV-2 can cause severe respiratory distress, particularly in immunocompromised individuals. However, unlike SARS-CoV and MERS-CoV, SARS-CoV-2 is highly contagious, very stable, with many person-to-person transmissions, which can occur even before individuals exhibit any symptoms. While vaccines are readily available, the emergence of new SARS-CoV-2 variants along with the increasing incidence of individuals developing long COVID urge to develop antivirals specific to treat COVID-19. To reach this goal, we need to have a working knowledge of the host–SARS-CoV-2 interactions to identify targets for therapeutic intervention.

Methods: Following that rationale, we focused on understanding how SARS-CoV-2 generates replication organelles (ROs). All coronaviruses need to remodel cellular membranes to create these structures to allow the active replication and transcription of their genome. Due to their relevance for virus replication, disabling RO formation represents a promising strategy to fight SARS-CoV-2. However, the biogenesis mechanism, the origin, and type of these replication organelles are still a major focus of debate. To identify the cellular membranes that SARS-CoV-2 uses to generate ROs we used multiple cell lines and primary cells that were evaluated by fluorescence microscopy, genetic engineering, compounds that specifically inhibit cellular processes, and immunoprecipitation assays to validate protein-protein interactions. We also used RT-qPCR to assess viral genome replication.

Results: SARS-CoV-2 uses the viral protein NSP6 to remodel endosomal membranes juxtaposed to the ER to generate replication organelles. Specifically, the virus depends on Clathrin, COPB1, and Rab5 for efficient SARS-CoV-2 RNA synthesis.

Conclusion: Uncovering the origins and mechanism(s) by which SARS-CoV-2 assembles ROs opens new avenues to develop strategies to interfere with RO biogenesis and halt virus replication.

WITHDRAWN
MPOX VIRUS SEQUENCES DIVERSITY IN PARIS AREA DURING THE 2022 OUTBREAK

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Background: Since May-2022, non-epidemic countries reported mpox cases with no epidemiological link to Africa, mostly affecting men-who-have-sex-with-men (MSM). We deciphered genetic variability of epidemic mpox from French infected patients and compared sequences with pre-epidemic Western-African sequences.

Methods: Sequences generated from 12 PCR-amplified fragments (~30,000 base-pairs) included outside terminal repeats, 28/35 mutation sites previously reported in epidemic isolates compared to reference clade-3 MT903344_2018 UK P2 pre-epidemic strain. All 7 unexplored mutations were synonymous.

Results: We studied 130 mpox-infected patients attending Bichat University-Hospital, Paris, France: 110 sampled during the early 2022 outbreak period (24/05-04/07) and 20 later on (16/08-10/09), from which we produced 162 sequences. Available medical records showed that 95.0% were men, 5% transgenders (M to F), and 90% MSM of whom 50% on HIV pre-exposure prophylaxis. Among these 25% lived with HIV and 25% had travelled within 3 weeks prior to mpox diagnosis (>85% to Europe, none to Africa); 5% were hospitalized, mostly for severe pharyngeal/tonsils. We observed 32 different mutational patterns (pattern#1 in 120 sequences; #9 in 10; and #13 in 2; others in 1). All included a 17nt-deletion (pos. 150,621, intergenic) previously observed in a French sequence (DN602722) but present in only 37% of epidemic strains. Pattern#8 included a previously unreported 4nt-deletion (pos. 14,509, ORF OPG25). Overall, 55% of single-nucleotide mutations were non-synonymous, 30% synonymous, 10% non-coding. There was a majority of transitions (15 G→A; 8 C→T), confirming a highly specific mutational typology.

In a patient returning from Asia, we identified a profile (pattern# 26), closely related to clade-3 pre-epidemic and N674051_2022 USA. This suggests a baseline circulation of non-epidemic mpox in Western countries, probably underdiagnosed and detected here due to attention generated by the outbreak.

Conclusion: This rapid parasimorous mpox sequencing strategy in 130 mpox-infected patients provided a unique insight into the genomic variability of an epidemic DNA virus, confirmed the existence of epidemic strain-specific mutational patterns and quantified the overall genomic variability of circulating strains. It highlights, although rare, the non-epidemic circulation of mpox and its immune evasion properties. Here, we investigated SARS-CoV-2 strain diversity in a French sequence (ON602722) but present in only 37% of epidemic strains. Beta and Delta dominated infections with B.1, B.1.1 and C.1 circulating at low levels.

Genomic Surveillance of SARS-CoV-2 Reveals Severity of Delta Variant in Cameroon

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*Presented at CROI by a nonauthor colleague

Background: At the global level, the dynamics of the COVID-19 pandemic have been driven by several epidemiological waves, determined by the emergence of new SARS-CoV-2 variants from the original viral lineage from Wuhan, China. While the SARS-CoV-2 pandemic has been described globally, there is a lack of data from Sub-Saharan African.

Methods: A laboratory-based survey was conducted in Cameroon, from March 1, 2020 to March 30, 2022, through an assessment of the evolutionary patterns of SARS-CoV-2 lineages across the four COVID-19 waves in the country. Data on full-length sequencing from all four sequencing laboratories were consecutively entered into the GISAID platform. These data were downloaded, and the molecular phylogeny of the SARS-CoV-2 sequences was performed using Nextstrain. The Mann–Whitney U test was used to calculate the correlation between the duration of each outbreak and the number of confirmed cases and between hospitalised cases and CFR, with a p value < 0.05 considered statistically significant.

Results: A total of 3,881 samples were successfully processed, of which 38.9% (n=1,509) using PCR mutation assay, 41.5% (n=1,611) using targeted sequencing, and 19.6% (n=760) using whole-genome sequencing. The mean age of the study population was 36 years (min–max: 2–86), and 45% were within the age range 26–45. Regarding gender distribution, 50.9% were male, and 49.1% were female. Phylogenetic analysis of the 760 whole-genome sequences generated from March 2020 to March 2022 revealed that the greater proportion of SARS-CoV-2 circulating in Cameroon belonged to the viral sub-lineages of the original strain from Wuhan (74%), 15% Delta variant, 6% Omicron variant, 3% Alpha variant and 2% Beta variant. The pandemic was driven by SARS-CoV-2 lineages of origin in Wave 1 (16 weeks, 2.3% CFR), the Alpha and Beta variants in Wave 2 (21 weeks, 1.6% CFR), Delta variants in Wave 3 (11 weeks, 2.0% CFR), and Omicron variants in Wave 4 (8 weeks, 0.73% CFR), with a declining trend over time (p=0.01208)
Conclusion: In a nutshell, the SARS-CoV-2 epidemic in Cameroon appears to have been driven by the SARS-CoV-2 lineage of origin in Wave 1, the co-introduction of the Alpha and Beta variants in Wave 2, the Delta variant in Wave 3, and the Omicron variant in Wave 4, with an overall declining trend in the wave duration, confirmed cases and hospitalisations over time. The SARS-CoV-2 lineage of origin and the Delta variant appeared to be the drivers of COVID-19 severity in Cameroon.

SARS-CoV-2 lineage dynamics per wave in Cameroon

240 EARLY EVOLUTION OF HIV-1 FROM TRANSMITTED FOUNDERS DURING ACUTE INFECTION

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Background: To better define the number of transmitted founder variants and sequence divergence from founders in the first few weeks following HIV-1 transmission, we performed ultra-sensitive single genome sequencing (SGS) of pol and env HIV-1 RNA in plasma samples from individuals with acute HIV-1 infection.

Methods: HIV-1 RNA was extracted from plasma samples of donors with acute CRF01_AE infection (Fiebig II–IV) enrolled in the RV254/SEARCH0101 Cohort (NCT00796146). Five of the donors had been identified by standard SGS as having multiple transmitted founders (TFs) and 10 as single TFs, consistent with the prior report showing 70% having single TFs in RV254. Our ultrasensitive SGS method with primer IDs and 500 bp paired-end Illumina sequencing was applied to identify >10,000 independent pol and env sequences per sample. We used radial trees (Figure 1) and star phylogeny to identify TFs, calculated transition/transversion (Ti/Tv) and non-synonymous/synonymous (dN/dS) ratios, identified drug resistance mutations (DRMs), and analyzed the phylogenetic relationships between emergent variants.

Results: An average of 12,823 pol and 11,249 env independent RNA sequences determined from consensus building of millions of barcoded reads were obtained from the plasma samples of the 15 donors. In single TF infections, an average of 85% of the genomes were identical to the TF. An average of 13% of the genomes contained only a single mutation. 1% of the genomes contained 2 mutations and 1% had 3–6 mutations, with 2 samples containing up to 12 mutations in a few genomes. Ti/Tv ratios were 5.5 and 3.4 in pol and env respectively. DRMs were found at very low frequency (0.01–0.09%) in all samples. Mutations predicted to confer resistance to the antibody VRC-01 were also present in all samples. The dN/dS in env (M=2.83, SD=0.90) was greater than pol (M=1.47, SD=0.74; t(19)=3.31, p=0.009). Mutation frequency was higher in both genomic regions in samples from later Fiebig stages.

Conclusion: Overall, ultra-sensitive SGS (>10,000 genomes per sample) did not identify more transmitted founder variants than low depth (10 genomes) SGS, verifying that HIV-1 infection is most commonly established by a single founder. By contrast, ultra-sensitive SGS revealed viral evolution from the founder variants in all samples including those from as early as Fiebig stage II, indicating rapid evolution of HIV-1, including appearance of DRMs soon after transmission.

Figure 1. Phylogenetic analyses of ultrasensitive SGS data on one donor previously identified by standard SGS as having a single founder (A) and one previously identified by standard SGS as having multiple founders (B). The red circles show the consensus sequences of the predicted transmitted founders. More detailed phylogenetic analyses will be presented.
provide an important foundation to study HIV pathogenesis in humans and design interventional measures to curb/reduce HIV transmission.

**Methods:** Full length infectious molecular clones of viruses were derived from people with acute HIV-1 clade C infection from IAVI Protocol C cohort. Two viruses, HIV-1K4790 and HIV-1K4791, were generated by plasmid DNA transformation, purification, and propagation in PBMCs. Donor-matched ecto- and endocervical explants derived from HIV-1-negative volunteers from Chicago and Nairobi, were then infected with HIV-1ExG, HIV-1K4790 and HIV-1K4791 using nevirapine as a control. The explants were then incubated at 37°C, 95% humidity and 5% CO₂ for 12 days. DNA was extracted and HIV-1 replication assessed by RT-qPCR.

**Results:** There was no significant differences in rate of infection between HIV-1ExG and HIV-1K4790 in the ectocervical tissues. However, HIV-1K4790 produced less virus in the ectocervix compared to HIV-1ExG (p<0.01). HIV-1K4790 had a lower rate of infection in the ectocervix compared to HIV-1ExG (p=0.03, n=36). In the endocervical tissues, HIV-1K4790 infected less compared to HIV-1ExG (p=0.03, n=36). However, the rate of infection between HIV-1ExG and HIV-1K4790 in the ectocervix was similar (p > 0.01). HIV-1K4790 had a lower rate of infection in the ectocervix compared to HIV-1ExG (p=0.03, n=36). In the endocervical tissues, HIV-1K4790 infected less compared to HIV-1ExG (p=0.03, n=36). However, the rate of infection between HIV-1ExG and HIV-1K4791 in the ectocervix was similar. Further, HIV-1K4791 and HIV-1K4790 replicated similarly in endocervix and ectocervix.

**Conclusion:** We demonstrate that the ectocervix and endocervix are differentially infected with TF HIV-1, indicating that these two compartments are distinct. In addition, we demonstrate a potential assay capable of testing potency of HIV antiviral drugs at mucosal surfaces ex vivo. In the next phase, using the same model, we will use imaging techniques to characterize the initial immune targets of HIV-1K4790 and HIV-1K4791 in the human cervix. Taken together, the discovery and characterization of TF HIV-1 replication in human mucosal explant cultures may serve as a crucial reference point for investigating HIV pathogenesis and developing interventional strategies to prevent HIV transmission.

**245 T CELLS HOMEOSTASIS DISTURBANCES IN A COHORT OF LONG-TERM ELITE CONTROLLERS**

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**Background:** Elite controllers (EC) are an exceptional group of people living with HIV (PLWH) able to control HIV replication without antiretroviral therapy. Further characterization of host responses and genetics in this unique group of HIV-infected individuals could reveal new insights into HIV extreme elite control.

**Methods:** Forty-three PLWH were included: 20 LTEC, 14 non-controllers cART-naïve with replicating HIV and 9 non-controllers cART, 9 non-controllers cART undetectable. Serum samples were collected from an occupational cohort in Guinea-Bissau. HIV-2 control was determined by quantifying plasma viral RNA load (pVL) and cell-bound viral DNA load. Immune modulation was analyzed by profiling of plasma and phenotyping of T-cell subsets, using markers previously linked to HIV disease progression and immune exhaustion.

**Results:** The HIV-2-infected individuals were stratified into the following groups: i) viremic with pVL above the pQPCR quantitation level (≥ 75 copies/mL); ii) low viremic with pVL above the detection level but below the quantitation level (5-75 copies/mL); iii) elite controllers (ECs) with undetectable pVL (< 5 copies/mL) and detectable cell-bound DNA load (≥ 1 copy/10⁹ leukocytes); iv) extreme elite controllers (EECs) with undetectable pVL and undetectable cell-bound viral DNA load (< 1 copy/10⁹ leukocytes). Thus, the EEC definition contrasts with that of HIV-1 EC, where cell-bound viral DNA is detected. Further characterization showed that HIV-2 ECs could not be distinguished from HIV seronegative individuals by neither CD4+ T-cell level, concentrations of plasma markers previously linked to HIV disease progression, nor frequencies of chronically activated and exhausted T cell populations. Moreover, plasma proteome profiling and unsupervised hierarchical clustering revealed that reduced plasma concentrations of soluble co-inhibitory receptors differentiated the EEC group from the other groups.

**Conclusion:** EECs represents a previously undefined group of HIV-2-infected individuals able to spontaneously control their HIV infection. Further characterization of host responses and genetics in this unique group of HIV-infected individuals could reveal new insights into HIV extreme elite control, and spur novel functional cure strategies.

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**References:**

1. Balic M, Ollerton M, Albert A, Rakasz E, Skinner P, et al. Follicular T helper cells (TFH) are the major viral RNA expressing cells in secondary lymphoid tissues during chronic HIV/SIV infection prior to AIDS/SADS. **TFH** are variably defined based on location in germinal centers (GC), and/or expression of the canonical transcription factor BCL6. TFH are variably defined based on location in germinal centers (GC), and/or expression of the canonical transcription factor BCL6. Whether BCL6+ cells provide an important foundation to study HIV pathogenesis in humans and design interventional measures to curb/reduce HIV transmission.

**Methods:** Full length infectious molecular clones of viruses were derived from people with acute HIV-1 clade C infection from IAVI Protocol C cohort. Two viruses, HIV-1K4790 and HIV-1K4791, were generated by plasmid DNA transformation, purification, and propagation in PBMCs. Donor-matched ecto- and endocervical explants derived from HIV-1-negative volunteers from Chicago and Nairobi, were then infected with HIV-1ExG, HIV-1K4790 and HIV-1K4791 using nevirapine as a control. The explants were then incubated at 37°C, 95% humidity and 5% CO₂ for 12 days. DNA was extracted and HIV-1 replication assessed by RT-qPCR.

**Results:** There was no significant differences in rate of infection between HIV-1ExG and HIV-1K4790 in the ectocervical tissues. However, HIV-1K4790 produced less virus in the ectocervix compared to HIV-1ExG (p<0.01). HIV-1K4790 had a lower rate of infection in the ectocervix compared to HIV-1ExG (p=0.03, n=36). In the endocervical tissues, HIV-1K4790 infected less compared to HIV-1ExG (p=0.03, n=36). However, the rate of infection between HIV-1ExG and HIV-1K4790 in the ectocervix was similar (p > 0.01). HIV-1K4790 had a lower rate of infection in the ectocervix compared to HIV-1ExG (p=0.03, n=36). In the endocervical tissues, HIV-1K4790 infected less compared to HIV-1ExG (p=0.03, n=36). However, the rate of infection between HIV-1ExG and HIV-1K4791 in the ectocervix was similar. Further, HIV-1K4791 and HIV-1K4790 replicated similarly in endocervix and ectocervix.

**Conclusion:** We demonstrate that the ectocervix and endocervix are differentially infected with TF HIV-1, indicating that these two compartments are distinct. In addition, we demonstrate a potential assay capable of testing potency of HIV antiviral drugs at mucosal surfaces ex vivo. In the next phase, using the same model, we will use imaging techniques to characterize the initial immune targets of HIV-1K4790 and HIV-1K4791 in the human cervix. Taken together, the discovery and characterization of TF HIV-1 replication in human mucosal explant cultures may serve as a crucial reference point for investigating HIV pathogenesis and developing interventional strategies to prevent HIV transmission.

**245 T CELLS HOMEOSTASIS DISTURBANCES IN A COHORT OF LONG-TERM ELITE CONTROLLERS**

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**Background:** Elite controllers (EC) are an exceptional group of people living with HIV (PLWH) able to control HIV replication without antiretroviral therapy and have been proposed as a model of functional HIV cure. However, several evidence suggest that this spontaneous control of HIV has a cost in terms of systemic inflammation and immune activation. Herein we have performed a deep phenotypic study to get insight into the T-cells homeostasis disturbances in EC maintaining long-term virologic and immunologic control of HIV (long-term elite controllers; LTEC).

**Methods:** Forty-three PLWH were included: 20 LTEC, 14 non-controllers under successful cART (TX), 9 non-controllers cART-naïve with replicating HIV (TP). Nineteen healthy participants (HC) were included as reference. T-cells homeostasis was analysed by spectral flow cytometry using a panel of 22
different T-cells markers. A minimum of 25,000 CD4 and CD8 T cells were acquired. Data were analyzed using an omic approach in R software. The analysis included batch correction, tSNE dimensionality reduction and Louvain clustering. Abundance of T-cells clusters was compared between groups by Mann-Whitney test and an adjusted p-value < 0.05 was considered as significant.

**Results:** Median follow-up maintaining virologic (undetectable plasma HIV load) and immunologic (stable CD4 counts) control in LRTC was 13 (7-16) years. Dimensionality reduction and clustering yielded 71 and 68 different CD4 and CD8 T cells clusters respectively. TP patients showed the highest level of T-cells disturbances with 21 CD4 clusters and 26 CD8 clusters significantly different from HC. Most of these alterations were reverted in TX who presented only 2 CD4 clusters and 1 CD8 cluster different from HC. Interestingly, LRTC presented a high level of CD4 and CD8 T-cells disturbances with 15 CD4 clusters and 23 CD8 clusters different from HC. Altered CD4 clusters included an increase of exhausted central memory cells and a decrease of naive cells and peripheral follicular T helper (pTfh) cells. Altered CD8 clusters included an increase in exhausted and senescent terminally differentiated cells, and a decrease of naive cells.

**Conclusion:** Our results suggest that, compared to cART-mediated control of HIV, the spontaneous control of HIV is associated with several disturbances in CD4 and CD8 T cells homeostasis. The impact of these alterations in both the loss of spontaneous HIV control and in the state of persistent systemic inflammation need to be further analyzed.

### 246 LOW TRYPTOPHAN CATABOLISM MARKS IMMUNE PRESERVATION IN HIV+ VIREMIC NON-PROGRESSORS

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**Background:** Viremic Non-Progressors (VNPs) is a rare subgroup of HIV+ individuals who maintain high CD4 counts despite high viremia in the absence of antiretroviral treatment (ART), resembling SIV infection in natural hosts.

The mechanisms underlying this phenotype are unclear; however, the VNP phenotype has been associated with lower expression of Interferon (IFN)-stimulated genes across multiple immune cells. Elevated IFN responses can alter host metabolism to impact immune homeostasis. However, the potential link between IFN responses, host metabolic alterations, and immune homeostasis in VNPs is unknown.

**Methods:** We retrospectively selected plasma samples from 16 VNPs and 29 HIV progressors with similar viral load (VL; median log VL > 4) but different CD4 decay rates (median annual CD4 loss < 10% and > 10%, respectively) before ART initiation. Plasma samples from six HIV-1 seronegative individuals (HIV-) were included as a reference. Soluble plasma markers of inflammation and microbial translocation were measured using ELISA and multiplex cytokine arrays. Untargeted plasma metabolomic analysis was performed by mass spectrometry. Metabolic pathway analysis was performed using MetaboAnalyst 5.0.

**Results:** Levels of the proinflammatory type II IFN (IFNy) were similar in VNPs and HIV-, but increased in Progressors (P < 0.05; Fig. 1A). However, no difference in the levels of the antiviral type I IFN (IFNα) was observed among the three groups. Levels of plasma IFNγ were strongly associated with an upregulation of tryptophan (Trp) catabolism and nicotinamide metabolism pathways (FDR=0.006 and P=0.038, respectively, Fig. 1B). Consistently, levels of metabolites within the Trp catabolism pathway (including anthranilic acid, quinolinic acid, and Q), and the ratio of Q/Trp, with established immunomodulatory roles, were increased in Progressors, but not in VNPs, compared to HIV-controls (Fig. 1C). Finally, high Trp catabolism correlated with high levels of proinflammatory cytokines and low CD4 counts (Fig. 1D).

**Conclusion:** Increased Trp catabolism, as observed in HIV Progressors, is known to restrain CD4 T-cell function and decrease T cell survival. Conversely, unaltered Trp metabolic profile was linked to immune preservation in VNPs. Unraveling these mechanisms may guide the development of novel therapeutic strategies to maintain immune homeostasis during HIV infection.

### 247 PHENOTYPE OF SIV-INFECTED CELLS IN BLOOD AND TISSUES USING A NEW SINGLE-CELL APPROACH

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**Background:** CD4+ T cells are the main cellular targets of both HIV and SIV. While the phenotype of HIV-infected cells has been extensively reported, the cellular subsets in which SIV actively replicates in blood and tissues from non-human primates (NHPs) remain largely unidentified. We developed a novel flow-based approach to identify the phenotype of SIV-infected cells during untreated SIV infection. By using two different antibodies specific for the p27 SIV protein (2F12 and 4B7) we enumerated and phenotyped productively infected cells with high specificity. The assay (SIV-Flow) can detect a single infected cell in a million CD4+ T cells and has a large dynamic range (1 to 100,000 infected cells/10⁶ CD4+ T cells).

**Methods:** We analyzed infected cells in multiple tissues from 4 SIVmac239-infected ART-naive rhesus macaques, including blood, spleen, axial, mesenteric and inguinal lymph nodes. CD4+ T cells were isolated by negative magnetic selection and stained with a combination of antibodies to obtain the phenotype of p27+ cells (CTLA-4, PD-1, Ki67, CD69, CD127, CCR7, CD27, CD95, CD3, CD4, CD8).

**Results:** In all animals, the highest frequencies of p27+ cells were measured in the spleen (mean 1887 cells/10⁶ CD4+ T cells), whereas blood and lymph nodes displayed similarly lower frequencies (842 and 848 cells/10⁶ CD4+ T cells, respectively). p27+ were under represented in the naïve compartment in both blood and tissues (fold enrichment of 0.6 and 0.3, respectively). In the blood, p27+ cells were highly enriched in the central memory subset (fold enrichment of 9.8), whereas p27+ cells frequently displayed a transitional and effector memory phenotype in lymphoid tissues (fold enrichment of 2.3 and 2.9, respectively).

Overall, when compared to their uninfected counterparts, p27+ cells expressed higher levels of several cellular markers including PD-1 (68% vs 32%), CD69 (47% vs. 23%), Ki67 (38% vs. 12%) and CTLA-4 (21% vs. 12%). Of note, enrichment of p27+ cells subsets expressing these activation and exhaustion markers was more pronounced in lymphoid tissues.

**Conclusion:** In untreated SIV-infected NHPs, productively-infected cells are found at higher frequencies in the spleen, which can be explained by the high percentages of PD-1+ and effector memory cells and the relatively low proportion of naive cells in this tissue. Our results suggest that activated CD4+ T cells in the spleen are important contributors to SIV replication in chronically infected NHPs.

### 248 CHANGES IN GUT MICROBIOTA PROFILE IN PWHIV WHO SWITCH FROM EFV/FTC/TDF TO BIC/FTC/TAF

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**Background:** Weight gain is often reported when virologically suppressed people living with HIV (PWHIV) switch to integrase inhibitors (INSTI). Reasons linking INSTI to weight gain are unclear and might be attributed to this HIV
drug class, to coformulation with tenofovir alafenamide, and/or influenced by medications, lifestyle and environmental factors. One factor that could impact weight gain is the gut microbiota. Ample evidence exists on the reciprocal relationship between the gut microbiome and host metabolism. Here, we evaluated changes in the gut microbiome in antiretroviral-treated PWHIV who switched from EFV/FTC/TDF to BIC/FTC/TAF.

Methods: This is a prospective single-center cohort study. A total of 27 PWHIV, 14.8% females (n=4), median age of 42 (26-53) years, virologically-suppressed (mean of 8.51 ± 4.98 years), were included. Fecal samples were obtained pre and one-year (mean of 367.3 ± 10.52 days) postswitch, and subjected to 16S rRNA sequencing.

Results: Alpha diversity (shannon) increased significantly postswitch (median 3.9 [3.5-4.1] compared to 2.8 [2.1-3.4] preswitch, p<0.0001), this increase was observed for both sexes. Bacteroidota dominated, averaging 57.43% abundance preswitch, decreasing to 47.10% postswitch, with a concurrent increase in Firmicutes abundance (27.16% to 43.12%). The Firmicutes to Bacteroidota ratio, a putative marker of obesity and weight gain, increased postswitch (median 1.024 [0.50-1.739] versus 0.34 [0.046-1.023] preswitch, p=0.02). At genus level, Prevotella (males) and Bacteroides (females) dominated, Faecalibacterium (both sexes) and UCG_002 (males) increased while Succinivibrio (both sexes) decreased postswitch. Principle-coordinate analysis revealed that microbial community clustering was mostly influenced by intra- and interpersonal variation (subjects) and sex, explaining 67.5% and 6.5% of the variance (PERMANOVA p=0.0001 and p=0.002, respectively).

Conclusion: The complex nature of weight gain associated with HIV drug classes remains to be elucidated, and might reflect contextual host-gut microbiota adaptations. The work presented here expands our understanding of weight gain associated with INSTI. Our findings suggest that switching to INSTI might modulate the gut microbiota, increasing its diversity and the abundance of Firmicutes in both sexes, favoring weight gain by affecting energy extraction. Long-term health implications and therapeutic avenues should be further investigated to prevent or manage weight gain on INSTI.

249 PROTEOMIC ANALYSIS OF TRANSLATING BACTERIAL TAXA REVEALS NOVEL THERAPEUTIC TARGET

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Background: Microbial translocation contributes to inflammation in HIV-infected humans and has been associated with increased mortality and morbidity in individuals treated with antiretrovirals. Thus, there is interest in understanding the mechanisms underlying microbial translocation. To characterize translocating bacterial populations, we isolated and identified translocating bacteria from chronically HIV-infected macaques and characterized their genomes and proteomes. Proteome profiling identified a cytosine-specific DNA methyltransferase as being enriched in translocating taxa. We hypothesized that inhibiting this enzyme with the cytidine analog 5-Aza-2'-deoxycytidine would more strongly inhibit the growth of translocating bacteria compared to non-translocating bacteria.

Methods: Liver, mesenteric lymph node, and spleen samples were taken during necropsy from twenty chronically HIV- or SHIV-infected RMs. Tissue samples were homogenized and plated under aerobic and anaerobic conditions. Isolates were identified using MALDI-TOF and/or 16S rRNA sequencing. Bacterial proteomes were analyzed by tandem mass spectrometry and genomics by next generation sequencing. 5-Aza-2'-deoxycytidine mediated growth inhibition was determined in vitro via longitudinal OD600 readings. Under the curve was used to determine inhibitory effects between species, Welch’s t test or Mann-Whitney test used to compare results depending on normal distribution.

Results: Thirty-six translocating bacterial taxa were identified from 4 bacterial phyla. Translocating bacteria showed different proteome features from non-translocating bacteria with 47.21% of proteins identified as being unique. Top hits included cytosine-specific DNA methyltransferases and copper homeostasis protein CufC, which were found in five of the eight translocating bacterial species. 5-Aza-2'-deoxycytidine preferentially inhibited the growth of translocating bacterial species compared to non-translocating species.

Conclusion: Microbial translocation does not seem to be stochastic and unique taxa of translocating bacteria commonly express DNA methylation enzymes. Inhibition of these enzymes in vitro results in significant reduction of growth in these taxa. Blocking activity of these enzymes in vivo may offer unique treatment modalities to reduce microbial translocation and improve the prognosis of HIV-infected individuals.

THE EFFECTS OF CD4+ LYMPHOPENIA ON THE HUMAN SKIN MICROBIOME

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Background: The role of specific immune components in maintaining host-microbial homeostasis remains poorly defined. People with HIV (PWH) and Idiopathic CD4 Lymphocytopenia (ICL) patients exhibit low CD4 T cells in distinct clinical contexts providing a unique opportunity to decipher the role of helper CD4 T cells in skin host-microbial interactions. These patients’ increased risk of comorbidities includes opportunistic fungal, mycobacterial and viral infections, neoplastic, and autoimmune or inflammatory skin disorders. We hypothesized ICL and PWH have prominent skin microbiome shifts, related to immune dysfunction and clinical comorbidities.

Methods: Skin microbiome topographical diversity (shinggun metagenomics) was analyzed in PWH, ICL and healthy controls (HCs).

Results: We collected skin samples from PWH naive to any antiretroviral therapy (ART) (PWH-naive, n=5, median CD4 48cells/mL, median plasma (p) HIV RNA=361206 copies/mL, ICL (n=8, median CD4 65cells/mL), and HC. PWH samples 2 (n=5) and 15 mos (n=4) after ART initiation were collected. HCs typically demonstrated shared microbiome features; PWH and ICL exhibited greater inter-individual heterogeneity in their microbiomes, consistent with prior studies in immunodeficiencies. As hypothesized, PWH and ICL had notable skin microbiome shifts: PWH-naive had higher fungal relative abundances, and ICL exhibited higher DNA viral relative abundances. Mean fungal relative abundances of inner forearm were significantly higher on PWH-naive than on ICL (18% and 3%, respectively; p=0.00002) and HCs (7%; p=0.00009), while mean relative abundances of viruses on PWH-naive were lower than those on ICL (4% and 5%, respectively; p=0.06). Interestingly, PWH-naive and HCs across all sites had significantly different microbiomes, compared to PWH on ART (n=4, median CD4 338cells/mL, median time on ART=15 mos, pHIV RNA < 40 copies/mL) and HCs (mean Bray-Curtis distances 0.52 and 0.39, respectively; p=0.004).

Conclusion: Our data suggest that CD4 T cell lymphopenia may differentially affect skin microbiomes and consequent clinical manifestations based on underlying immune deficiency. ART plays a pivotal role in shifting PWH skin microbiomes towards HCs. Studying human skin microbiomes in such contexts can provide insights into susceptibility to microbial diseases (i.e. viral vs fungal) as well as the interactions between the skin microbiome and host immune deficiency and reconstitution.

Skin microbiomes of HIV, ICL, and healthy controls
**COMMENSAL BACTERIA WITHIN THE FAMILY LACHNOSPIRACEAE ROBUSTLY INHIBIT HIV INFECTION**

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**Background:** The microbiota, the vast collection of microorganisms residing in our body, regulates the susceptibility to and severity of various infectious diseases. In HIV infection, HIV-induced dysbiosis of the gut microbiota is well-recognized. However, it is less clear whether the gut microbiota—and which specific microbes and microbial mechanisms—impacts susceptibility to HIV.

**Methods:** To identify bacterial taxa associated with HIV susceptibility, we analyzed 16S rRNA gene sequences generated from stool of infant nursery-reared rhesus macaques collected before they were orally challenged with SHIV. For bacterial taxa identified to be associated with protection against SHIV, we obtained the corresponding human-derived isolates and experimentally validated their inhibitory effects on HIV pathogenesis by treating TZM-bl reporter cells with heat-killed bacteria before infecting with HIV. Additionally, we performed time-of-addition assays to identify the specific steps of HIV replication targeted by these bacteria.

**Results:** We bioinformatically identified two bacterial taxa, Lactobacillus gasseri and the family Lachnospiraceae, that are associated with protection against HIV. Although strains of L. gasseri demonstrated variable inhibitory effects against HIV infection, Clostridium immunis and Ruminococcus gravis, two species within the family Lachnospiraceae, robustly inhibited HIV infection of TZM-bl cells. Based on time-of-addition assays, C. immunis inhibited viral entry and reverse transcription, while R. gravis inhibited integration in addition to these steps. Given that tryptophan metabolism is known to be critical in regulating the severity of HIV infection, we developed bacterial genetics for C. immunis and inactivated the amino acid aminotransferase (ArAT) gene, which metabolizes tryptophan into 3-indolelactic acid. Intriguingly, C. immunisΔArAT was a less potent inhibitor of HIV infection compared to its wild-type parental strain, a finding that indicates the ArAT gene is critical for inhibition of HIV.

**Conclusion:** We bioinformatically identified and experimentally validated three different commensal bacterial species as being able to inhibit HIV infection. Moreover, we established that metabolism of tryptophan by a commensal bacterium impacts HIV infection. More broadly, our results provide mechanistic insight into how commensal bacteria affect HIV pathogenesis and may inform new therapies for the prevention of HIV.

**252 BACTERIAL DYSBIOSIS PERSISTS IN WOMEN LIVING WITH HIV-1 AFTER ART**

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**Background:** The cervicovaginal (CV) microbiome has a critical role in women’s health and disease, including HIV acquisition and progression. A healthy cervicovaginal (CV) microbiome is dominated by a single Lactobacillus species that helps maintain a low vaginal pH, prevent outgrowth of harmful bacteria, and regulate immunomodulation. Bacterial vaginosis (BV) occurs when Lactobacillus is depleted and replaced by an outgrowth of facultative or obligate anaerobes. Women living with HIV (WLWH) have high prevalence of BV- associated bacteria that are linked to vaginal inflammation, higher genital HIV viral loads, and greater HIV shedding. However, the impact of antiretroviral treatment (ART) on the CV microbiome has not been described in depth. In this study, we sought to evaluate the CV microbiome of ARV-naïve WLWH over their first year of ART.

**Methods:** CV pellets were collected longitudinally from 83 ART-naïve cisgender Ugandan WLWH at week 0, 8, 24, and 52 of ART. After DNA extraction, the V3-V4 region of the bacterial 16S rRNA gene was amplified by PCR and sequenced using an Illumina NovaSeq platform. Paired end FASTQ files were analyzed using the DADA2 package in R. Reads were binned into amplicon sequence variants (ASVs) and the SILVA taxonomic framework were used to taxonomically annotate them. ASVs were analyzed using the Phyloseq and DeSeq2 packages in R.

**Results:** The median age of participants was 31 years and median HIV plasma viral load was 4.4 log10 copies/ml with a CD4 count of 400 cells/ml. ART resulted in a restored median CD4 count of 687 cells/ml after 52 weeks (p = 0.0001) and undetectable viremia in 86% of participants (43/50). Gardnerella was identified as the most abundant family in our dataset with a median relative abundance of 35% at week 0. Gardnerella maintained a high prevalence throughout the study with a median relative abundance of 46, 35, and 35% at week 8, 24, and 52, respectively. Bacterial diversity, measured by Shannon Diversity, at week 52 did not differ from week 0 (p = 0.91), although seven genera were significantly less abundant at week 52 compared to baseline including the BV-associated bacteria Peptostreptococcaceae (log2FC = -4.01, p < 0.0001) and Prevotellaceae (-1.09, p = 0.01).

**Conclusion:** Our findings indicate that ART alone does not effectively modulate compositional diversity of the CV microbiome, however, abundance of some BV-specific bacteria was reduced following treatment. These data support exploration of targeted therapeutics to restore the CV microbiome.

**BODY MASS INDEX AND INFLAMMATION IN PEOPLE LIVING WITH HIV AND UNINFECTED CONTROLS**

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**Background:** In the present study we aimed to explore differences in inflammatory markers between people living with HIV (PLWH) and uninfected controls before and after stratification according to body-mass-index.

**Methods:** 710 well-treated PLWH and 86 uninfected controls where included from the Copenhagen Comorbidity in HIV infection (COCOMO) study. Study participants were stratified into overweight/obese and normo-/underweight according to their BMI (< 25 and ≥ 25, respectively). Hypotheses were tested by linear and logistic regression analyses adjusted for age and sex.

**Results:** PLWH had lower BMI compared to uninfected control (25.1 vs 26.2, p-value 0.004), but no difference in prevalence of overweight/obesity was found between the two groups (43% vs 53%, p-value 0.086). While no differences in IL-6, IL-10, IFN-γ and hs-CRP were found, PLWH had higher levels of TNF-α (7.9 (4.6) vs 6.7 (2.6), p-value 0.04) compared to uninfected controls. After stratification according to BMI and adjustment for confounders HIV infection was associated with excess risk of high levels of IL-6 (aOR 5.82 [1.69 – 20.05]) and IFN-γ (aOR 3.41 [1.01 – 11.46]) in normoweight/underweight, but not among overweight/obese individuals (aOR 2.03 [0.91 – 4.55] and aOR 2.46 [0.91 – 6.49], respectively). When restricting the analyses to normoweight/underweight individuals, age, smoking and waist-to-hip ratio were associated with higher concentrations of IL-6 in PLWH, but not in uninfected controls (Table 1).

**Conclusion:** In the present study, HIV was associated with higher levels of IL-6 and IFN-γ in normoweight/underweight but not in overweight/obese individuals. Interestingly, waist-to-hip ratio was a predictor of higher IL-6 in normoweight PLWH and not in uninfected controls. Taken together,
our results may suggest a pivotal role for adipose tissue deposition in the early establishment of a pro-inflammatory milieu, even in individuals not clinically overweight or obese. Given the well-known association of systemic inflammation with non-AIDS associated cardio metabolic comorbidities, our results may suggest the need of more intensive monitoring for comorbidities in a subgroup of PLWH otherwise described at low risk.

Predictors of IL-6 and Interferon-γ levels in PLWH and uninfected controls after stratification according to BMI

### Table 1 – Predictors of IL-6 and Interferon-γ levels in PLWH and uninfected controls after stratification according to BMI

<table>
<thead>
<tr>
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<th>Normal weight/underweight</th>
<th>Overweight/obese</th>
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<tbody>
<tr>
<td><strong>Age, per year</strong></td>
<td></td>
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<tr>
<td>PLWH</td>
<td>0.95 [0.80-1.14]</td>
<td>0.97 [0.84-1.14]</td>
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<tr>
<td>Uninfected controls</td>
<td>0.96 [0.83-1.11]</td>
<td>0.98 [0.86-1.13]</td>
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<tr>
<td><strong>Sex, male vs female</strong></td>
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<tr>
<td>PLWH</td>
<td>0.56 [0.44-0.71]</td>
<td>0.62 [0.49-0.78]</td>
</tr>
<tr>
<td>Uninfected controls</td>
<td>0.90 [0.75-1.09]</td>
<td>1.02 [0.87-1.18]</td>
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<tr>
<td><strong>BMI, per 1 kg/m² change</strong></td>
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<tr>
<td>PLWH</td>
<td>0.65 [0.54-0.79]</td>
<td>0.82 [0.65-1.02]</td>
</tr>
<tr>
<td>Uninfected controls</td>
<td>1.10 [0.92-1.31]</td>
<td>1.18 [0.99-1.39]</td>
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<tr>
<td><strong>Smoking, yes vs no</strong></td>
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<tr>
<td>PLWH</td>
<td>0.56 [0.44-0.71]</td>
<td>0.62 [0.49-0.78]</td>
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<td>0.90 [0.75-1.09]</td>
<td>1.02 [0.87-1.18]</td>
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**Table 2 – Levels of iron metabolism markers and their associations, in young, HIV, elderly ferropenic subjects**

<table>
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<tr>
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<th>Normal-weight/underweight</th>
<th>Overweight/obese</th>
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<tr>
<td><strong>Hepcidin</strong></td>
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<tr>
<td>PLWH</td>
<td>0.05 [0.03-0.09]</td>
<td>0.05 [0.03-0.08]</td>
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<tr>
<td>Uninfected controls</td>
<td>0.05 [0.03-0.08]</td>
<td>0.05 [0.03-0.08]</td>
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<tr>
<td><strong>sTfR</strong></td>
<td></td>
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<tr>
<td>PLWH</td>
<td>1.10 [0.86-1.40]</td>
<td>1.20 [0.92-1.56]</td>
</tr>
<tr>
<td>Uninfected controls</td>
<td>1.01 [0.78-1.30]</td>
<td>1.20 [0.92-1.56]</td>
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**Figure 1** – Transferrin receptor axis. A, Levels of iron metabolism-related parameters (Iron, Tf, sTfR, and ferritin) in Young non-HIV (Y), HIV, and Elderly non-HIV (E) groups. B) Hepcidin levels in Y, HIV, E and F (ferropenic with ferritin ≥ 50 ng/ml) groups. Comparison was assessed using non-parametric Kruskal-Wallis (KW) tests. Variables with a p-value < 0.05 were considered statistically significant. Grey lines represent minimum and maximum standard values. C) Association between sTfR and hepcidin and the rest of iron metabolism parameters in Y, HIV, and F groups. Color intensity of boxes represents Spearman’s rank correlation coefficient value as indicated in the color legend. All boxed boxes represent statistically significant correlations, except for those related to Y (p = 0.06 - p = 0.15). White boxes represent correlations with p-values < 0.1: Y, p = 0.02; E, p = 0.10; F, p = 0.09. Abbreviations: Tf, transferrin; Tsf, transferrin saturation index; sTfR, soluble transferrin receptor.

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**IRON METABOLISM IN CHRONIC HIV: IMPAIRED HEPcidin-SOLUBLE TRANSFERRIN ReCEPTOR AXIS**

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**Background:** Chronic HIV-infected subjects under effective antiretroviral treatment (ART) exhibit an immune dysfunction leading to comorbidities. Since iron metabolism plays an essential role in immune cell function, its regulation may be relevant in this context.

**Methods:** We included 92 asymptomatic chronic HIV-infected subjects under suppressive treatment (HIV), 25 age-matched non-HIV healthy donors (< 65 years old; Young, Y), and 25 non-HIV elderly subjects (> 65 years old; Elderly, E). For partial analysis, 84 patients with iron deficiency and ferritin< 50 ng/mL were also included (Ferropenic, F). Traditional biomarkers of iron metabolism, as well as soluble transferrin receptor (sTfR), hepcidin, and inflammatory markers were also included (Ferropenic, F). Traditional biomarkers of iron metabolism, as well as soluble transferrin receptor (sTfR), hepcidin, and inflammatory markers were also included (Ferropenic, F).

**Results:** Compared to both healthy (Young and Elderly) controls, HIV subjects exhibited decreased iron (Y, 100 [60-164]; HIV, 31 [24-42]; E, 81 [49-119] µg/dL), transferrin saturation (Y, 26 [16-29]; HIV, 11 [8-15]; E, 26 [15-37] %), and sTfR (Y, 3.1 [2.7-4.1]; HIV, 2.0 [1.5-2.6]; F, 3.9 [3.0-5.2] mg/L). We observed that sTfR is increased in HIV. Moreover, hepcidin levels were higher in HIV subjects (Y, 72 [25-142]; HIV, 119 [61-183] ng/mL), but similar hepcidin levels in HIV and Ferropenic groups (Figure 1A and B). As expected, ferropenic subjects showed the lowest levels of hepcidin (Y, 4.6 [1.1-14.4]; F, 1.9 [1.6-3.0] ng/mL). Interestingly, HIV showed altered relationships between iron parameters and their regulators unlike young, elderly, or even the ferropenic group (Figure 1C). Specifically, associations between sTfR and iron were negative in healthy and ferropenic groups, while positive in HIV. Moreover, the observed negative correlation between hepcidin and sTfR, observed in healthy and ferropenic groups, was absent in HIV. Interestingly, the HIV inflammatory profile differed from the Elderly one (with an inflammaging-related profile), showing high levels of homocysteine (HIV, 3.2 [2.4-4.3] vs. Y, 1.8 [1.6-2.2] mg/L; p < 0.0001) and β2-microglobulin (HIV, 2.1 [1.9-2.5] vs. Y, 1.8 [1.6-2.3] mg/L; p = 0.018) compared to young.

**Conclusion:** Chronic HIV-infected patients under ART exhibit an imbalanced regulation of iron metabolism-related components, including hepcidin and sTfR, suggesting a complex functional iron deficiency that could contribute to their persisting immune dysfunction.

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**ENDOTHELIAL GM-CSF IN PLWH CONTRIBUTES TO PROINFLAMMATORY VASCULAR MICROENVIRONMENT**

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**Background:** People living with HIV (PLWH) show clinical and radiological findings of persistent endothelial dysfunction (ED), and two- to higher incidences of critical cardiovascular emergencies which is independent of other general risk factors. The effects of endothelial derived granulocyte macrophage colony stimulating factor (GM-CSF) on infiltrating immune cells that infiltrate the endothelium in PLWH are unknown, as is the role of bacterial translocation on GM-CSF expression. Here, we investigated the quantitative changes of toll-like receptor-4 (TLR4) and GM-CSF expression in the vascular endothelium in PLWH to understand the biological significance of TLR ligands and endothelium-derived GM-CSF.

**Methods:** Immunofluorescence microscopy and digital image data quantification, FISH, RNA-Seq Western blot, and Standard Statistical tests using GraphPad Prism v6.02.

**Results:** Using immunofluorescence microscopy of primary human aortic endothelial cells (ECs) and of arterial tissue microarray slides from PLWH, SIV/SHIV-infected Rhesus macaques (RMs), and respective uninfected controls, we confirmed that GM-CSF and TLR4 are detectable in primary human aortic ECs and in aortic tissues irrespective of age or gender variability. In our ex vivo studies, the TLR4 ligand LPS induced EC expression of GM-CSF (Fig. 1A). In addition, we observed overall increased TLR4 on the aortic endothelium of SIV/SHIV infected RMs and PLWH, compared to uninfected controls (Fig. 1B, 1C).

**Conclusion:** Inadequate intestinal mucosal barrier function is responsible for bacterial translocation in PLWH. Detection of increased GM-CSF and TLR4 in the endothelium of PLWH or in SIV/SHIV infected RMs indicate that ED in HIV infection would potentially create a perpetuated supportive microenvironment for the infiltrating inflammatory immune cells. This work suggests that suppressing endothelium-derived GM-CSF might be an important direction for the development of future therapeutics to combat chronic ED in PLWH.
ENDOTHELIAL GM-CSF IN PLWH CONTRIBUTE TO PROINFLAMMATORY VASCULAR MICROENVIRONMENT

Figure 1: A. Endothelial cells express GM-CSF. B. Significantly high GM-CSF in the endothelium of atherosclerotic plaque (n = 18). C. High TLR4 expression in the vascular endothelium of SIV/HIV infected rhesus macaque (left: n = 37), and the endothelium of PWHI (right: n = 22).

256 EFFECT OF P. FRAGILE COINFECTION ON ART AND IMMUNITY IN SIV-INFECTED RHEUS MACAQUES

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Background: HIV and malaria are endemic in similar geographical areas. Previous work indicates that co-infection accelerates disease pathogenesis is unclear. We hypothesized that P. fragile co-infection of ART-treated, SIV+ rhesus macaques (RMs) would result in elevated viral load (VL), decreased CD4+ T-cell counts, and increased neutrophil frequencies.

Methods: Male RMs (n=4) were intravenously inoculated with SIVmac239 (TCID50=50); initiated daily ART at Week 10 (W10) post-SIV infection (post-SIV); were i.v. inoculated with P. fragile (20x10^6 infected erythrocytes [iRBCs]) at W12 post-SIV; and were treated with anti-malarials at W14 post-SIV. Plasma VL and peripheral parasitemia were monitored using qPCR and Giemsa-stained blood smears, respectively. CD4+ T-cell absolute counts and neutrophil frequencies were assessed via flow cytometry.

Results: Peak VL (median=9.92x10^6 RNA copies/ul) was reached by W3 post-SIV. Upon ART initiation, VLs decreased, with 2/4 RMs becoming undetectable by W12 post-SIV. Within two weeks of P. fragile inoculation, parasitemia reached peak levels (W12 post-SIV median % parasitemia=25.5% iRBCs), which declined after anti-malarial treatment. At W13 post-SIV, RMs exhibited detectable VLs, which were sustained until W17 post-SIV (median=9.34x10^4 RNA copies/ul). Compared to baseline (median=306.905 cells/ul), CD4+ T-cell counts declined by W8 post-SIV (median=140.5 cells/ul), increased after ART (median=193.5 cells/ul), followed by a sustained decline (median=173.59 cells/ul) after P. fragile inoculation. Neutrophil frequencies non-significantly increased at W14 post-SIV (median=71%), as compared to baseline (median=56.65%) and W3 post-SIV (median=55.35%).

Conclusion: In this pilot study, our observations suggested that P. fragile co-infection lowered ART efficacy, characterized by elevated SIV viral suppression and poorer CD4+ T-cell reconstitution. Additionally, we observed an increase in neutrophils after co-infection as compared to both baseline and peak SIV infection, potentially implicating a role for neutrophils in accelerated pathogenesis during co-infection. More work will be needed to confirm and characterize the mechanisms by which neutrophils may contribute to disease pathogenesis during SIV/malaria co-infection.

257 INITIATE IMPAIRMENT DURING SIV INFECTION ALTERS ZIKV VIRAL PATHOGENESIS

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Background: Flaviviruses are circulating in >100 countries across the globe and in areas with endemic HIV. Despite the occurrence of large flavivirus outbreaks and geographic overlap in countries with high HIV prevalence, there is insufficient research to determine the risks of flavivirus co-infection in people living with HIV and impact on co-morbidities. Here, we establish and define a model featuring SIV-infected pigtail macaques with ZIKV co-infection. We investigated the hypothesis that enhanced ZIKV pathogenesis occurs in people living with HIV.

Methods: Pigtail macaques (n=7) were infected with SIVmac239M and co-infected with ZIKV at 9 weeks post-SIV infection (SIV+/ZIKV+). Animals were compared to control animals (n=7) only infected with ZIKV (SIV+/ZIKV-). SIV and ZIKV viral loads in plasma and peripheral tissues were measured by qRT-PCR. Blood/peripheral blood mononuclear cells (PBMCs), lymph node, and rectal tissues collected pre- and post-SIV and/or ZIKV infection were evaluated for innate and adaptive immune responses by flow cytometry. Alterations in host responses to infection were measured in PBMC using NanoString gene expression analysis.

Results: At the time of ZIKV co-infection, SIV+ animals had severe CD4 depletion and had reached viral setpoint. ZIKV was detected on average for 1.43 days longer in SIV+/ZIKV+ animals in comparison to SIV−/ZIKV− control animals and peak viremia was shifted on average 1.81 days later in SIV+/ZIKV+ animals. ZIKV viral dissemination to tissues was less evident the SIV−/ZIKV− animals. Post-ZIKV infection, peripheral recruitment of CD16+ monocytes, the in vivo blood target of ZIKV infection, was dampened and delayed in SIV−/ ZIKV− vs SIV−/ZIKV+ animals and corresponded to the delayed ZIKV viremia in SIV−/ZIKV+ animals. Furthermore, PBMC gene expression analysis post-ZIKV infection revealed sustained and hyperactivation of the innate immune response, including interferon-alpha signaling, in SIV+/ZIKV+ animals.

Conclusion: Here, we provide evidence that ZIKV viremia is delayed and more persistent in SIV-infected macaques. Characterization of the host response revealed chronic innate immune activation and constitutive type I IFN signaling, concomitant with impaired tissue recruitment of innate immune cells during SIV/ZIKV co-infection. Collectively, these findings suggest that untreated SIV-HIV infection could create an environment of immunological tolerance that may lead to poor ZIKV viral clearance and promote pathogenesis.

258 LYMPHOID TISSUE VOLUMES AND GLUCOSE METABOLISM IN EARLY SIV INFECTION OF AGM AND RM

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Background: Early establishment of an effector function following viral infection has been proposed as a major driver of viral reservoir control in nonpathogenic SIV infection. The capability of lymphoid tissues (LT) to promptly expand following antigen presentation (LT elasticity) has not been studied in natural and non-natural hosts of SIV infection. In this study, we investigated changes in LT volumes and tissue glucose metabolism in African Green Monkeys (AGMs) and Rhesus Macaques (RMs) during the early days following SIV infection, using 18F-Fluordeoxyglucose (FDG)-Positron Emission Tomography (PET) Computed Tomography (CT).

Methods: Six AGMs and 5 RMs were inoculated intravenously with 300TCDS0 SIVmac and 300-1000TCDS0 SIVmac viruses, respectively. Whole-body PET/CT scans were administered at baseline, Day 3, 7, 10, 14, 21, and 42 p.i. LT volumes of interest and radiotracer uptakes (reported as standardized uptake value, SUV) were obtained by two independent operators by drawing regions in MIM software. Plasma viral loads and peripheral blood CD4+ T-cell counts were monitored throughout the follow-up.

Results: Plasma viremia at each timepoint, peak, and area under the curve (AUCd42) was similar between AGM and RM groups. Based on the CD4 count crash at Day 42 (20-80% drop, P<0.05) and all available follow-up (5-19 months p.i.), all RMs displayed normal disease progression (>65% CD4 drop at nadir). Starting at Day 3 p.i., a statistically significantly higher expansion of LTs was observed in the AGM group (Figure 1A, B, and C; P<0.05 (red star), P<0.1 (black star)). Consistently, AUCd42 of the relative expansion of LTs was higher in the AGM group (Figure 1D). Compared to baseline, tissue glucose metabolism levels in LTs at Day 42 p.i. were statistically significantly higher in both groups (P<0.05), with a higher increase observed in the AGM group (P<0.05 for spleen and inguinal lymph node (ILN)).

Conclusion: PET/CT imaging during the early days of SIV infection revealed a prompter expansion of LTs in the AGM group compared to the RM group, accompanied by an increase in tissue glucose metabolism. Future studies will focus on mechanisms responsible for LN expansion contraction (e.g. molecular pathways governing the elongation of fibroblastic reticular cells) as well as on longer follow-up of LT volumetric analyses coupled with 18F-FDG-PET imaging to suggest alternative functional cure strategies.
259 THE BREATH AND POLYFUNCTIONALITY OF T CELL RESPONSES TO CMV DIFFER BY HIV STATUS

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Background: Chronic cytomegalovirus (CMV) infection elicits large T cell responses with significant biological and clinical implications (chronic inflammation and frailty) in HIV infection. Such T cell responses have been shown to target a wide range of CMV proteins. Building on our prior work, we postulated that the breadth and polyfunctionality of T cell responses to an extensive panel of CMV antigens would differ by HIV status.

Methods: CD4 and CD8 T cell responses to peptide pools spanning 19 CMV open reading frames (ORFs) were measured by intracellular cytokine staining for production of IFN-γ, TNF-α and IL-2 in 42 men (20 HIV- and 22 virologically suppressed HIV+) in the Multicenter AIDS Cohort Study. Polyfunctionality of T cell responses was assessed by mean polyfunctionality index of responding cells (ranging from 0.33 for cells producing 1 cytokine and 1.00 for cells producing 3 cytokines). Differences between HIV+ and HIV- men were assessed by non-parametric Mann-Whitney test. Differences between bivariate Spearman’s correlation coefficients were assessed by Fisher’s Z test, and differences in slope of regressions by least-square linear regression.

Results: Median numbers of CMV ORFs triggering CD8 T cell responses in HIV+ men were significantly higher than in HIV- men (10 vs. 7, respectively; p = .04). This was primarily due to an excess of responses to late-expressed CMV antigens in HIV+ men. In HIV- men, the numbers of ORFs responded to were strongly and positively correlated with total T cell responses for both CD4 and CD8 T cells (r = 0.73, p < .001, and r = 0.88, p < 0.00001, respectively); these correlations were present but weaker in HIV+ men (r = 0.56, p = .007, and r = 0.59, p = .004, respectively; p = .04 for the difference in correlation in CD8 responses by HIV status). Slopes of the regression of total CD4 responses against the number of CD4 responses were lower in HIV+ men than in HIV- men (p = .06). Polymorphism of CD4 responses to CMV ORFs was significantly lower in HIV+ men than in HIV- men (mean PI = .33 vs. .59, respectively; p < .001).

Conclusion: The breadth of CMV-specific CD8 T cell responses was higher and its correlation with the total magnitude of these responses was weaker in HIV+ men than in HIV- men. These results, along with the lower polymorphism of CD4 responses and the greater response to late CMV antigens in HIV+ men, suggest that immune control of CMV infection may be different in HIV+ men receiving antiretroviral therapy than in HIV- men with similar lifestyle.

260 GLYCOMIC BIOMARKERS OF BIOLOGICAL AGING ARE ALTERED DURING SUPPRESSED HIV INFECTION

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MACS/WIHS Combined Cohort Study

Background: In the general population, host glycomic alterations—including loss of galactose (agalactosylation; measured as high levels of G-terminal ratio and G0 glycan groups)—on circulating IgGs drive inflammation and precede the onset of premature aging-associated diseases. However, the impact of antiretroviral therapy (ART)-suppressed HIV infection on these glycomic biomarkers of biological aging is unknown.

Methods: Using capillary electrophoresis, we analyzed the IgG glycomes of 988 samples from participants of the MACS/WIHS Combined Cohort Study: 237 HIV-negative men and 254 HIV- ART+ men (age/race matched); 254 HIV-negative women and 243 HIV+ ART+ women (age/race matched). HIV+ individuals were ART suppressed for ≥5 years with undetectable viral load and CD4 count of ≥ 350 cells/mm3. We also analyzed the IgG glycomes of 112 samples from HIV- and HIV+ ART+ male cases and controls; cases had coronary stenosis ≥50% in ≥1 coronary segments and controls had no coronary plaque (by CT angiography). Soluble markers of inflammatory aging (based on PMID:34888528) were measured using multiplex cytokine arrays on a subset of 400 samples. Comparisons between women and men were performed using multivariate models adjusting for age, race, and body mass index.

Results: Treated HIV infection was associated with pro-biological aging glycomic alterations; induction of the pro-inflammatory agalactosylated (false discovery rate (FDR) < 0.05; Fig.1A) and bisected GlcNAc glycans in both women and men, and a reduction of the anti-inflammatory sialylated glycans in men. Compared to men, women exhibited higher levels of the pro-inflammatory agalactosylated glycans (FDR < 0.05; Fig.1A) and lower levels of the anti-inflammatory sialylated glycans. HIV infection accelerated the pace of age-associated agalactosylation compared to HIV- controls (P < 0.02, Fig.1B). HIV-associated IgG agalactosylation correlated with higher inflammatory markers of aging (including CXCL9 and IP-10), especially in HIV+ ART+ women (P < 0.05; Fig.1C). Finally, agalactosylated glycans were higher in HIV+ men with coronary stenosis compared to controls (FDR < 0.05; Fig.1D).

Conclusion: Sex-dependent premature-aging-associated glycomic dysregulation is accelerated during ART+ HIV infection and is associated with inflammatory aging (inflammaging) and subclinical atherosclerosis. These HIV and/or ART-promoted inflamminaging-associated glycomic alterations warrant further investigation to examine their potential prognostic and functional significance.

Figure 1: Sex-dependent premature-aging-associated glycomic dysregulation is accelerated during ART+ HIV infection and is associated with inflammatory aging (inflammaging) and subclinical atherosclerosis. These HIV and/or ART-promoted inflamminaging-associated glycomic alterations warrant further investigation to examine their potential prognostic and functional significance.
Sex-based differences affect the natural and treated history of HIV infection and immune responses. We previously described that women undergoing reproductive aging have a progressive increase in inducible HIV reservoir size in men. Here we investigate possible underlying immunologic mechanisms.

Methods: Longitudinal stored samples (N=422) from virally suppressed cisgender women (N=60) and age-matched men (N=31) were identified from the AIDS Clinical Trials Group Longitudinal Integrated Randomized Trials (ALLRT). Selected participants were between the ages of 40-53 at the time of antiretroviral treatment (ART) initiation and virally suppressed (<20 cp/ml) throughout the study period. Participants did not report taking any hormonal therapy. At each timepoint we measured concentrations of 39 cytokine/chemokine levels using an Luminex platform. Cytokines with <70% of values below the limit of detection across all participants and time points were included (N=25). Cytokines were clustered based on trajectories using the kmShape package and the Frechet distance method. Within each cluster of cytokines, we fit a longitudinal mixed effects model to investigate the effect of sex on their trajectory.

Results: At baseline, median (range) CD4+ were 210 (3,659) cells/ml for women and 238 (7.5, 450) for men. Median follow up range (52, 230) months for women and 83 (28, 179) for men. Cytokines were clustered into 4 groups with distinct trajectories. The average cytokine value in cluster 1 (n=13 cytokines) remained relatively stable throughout the study period, while cluster 2 (n=3) showed high values at baseline with a rapid decline, cluster 3 (n=5) showed a steady decrease, and cluster 4 (n=4) showed low values at baseline with a steady increase over time (Figure 1). Two clusters showed significant sex differences in their trajectories. Specifically, cluster 1 (which included multiple proinflammatory cytokines, including markers of monocyte activation) and cluster 3 (which included the T cell homoeostatic factor IL-7) started higher in men but declined significantly slower in women during follow up.

Conclusion: Sex-specific features to the inflammatory response could influence HIV reservoir formation, latency maintenance and reversal. Persistent increased levels of IL-7 and proinflammatory cytokines in women suggest that homeostatic proliferation may have a differential contribution to HIV reservoir maintenance in female during aging.

Longitudinal clustering of cytokines based on trajectories over time

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Aging in Women with HIV: Single-Cell Transcriptome Analysis of the Immune System

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Background: Reproductive aging in women with HIV (WWH) introduces significant therapeutic challenges due to the progressive decline in blood levels of estrogen, a known negative regulator of HIV transcription. Our group recently described how the inducible HIV RNA reservoir, as measured by the EDITS assay, increased progressively in women during reproductive aging (PMID: 34612493). Here, we explore possible underlying mechanisms with detailed studies at a single-cell level, which will account for the heterogeneity of the immune cell populations.

Methods: We selected biorepository samples carefully staged across reproductive stages from cisgender women with HIV from the Chicago Women’s Interagency HIV Study (WIHS). Specifically, we first selected cross-sectional samples from eight WWH, four pre- and postmenopausal. To account for donor variability, we further selected 3 longitudinal samples (pre-, peri-, and postmenopausal) from 2 WWHs. Blood samples were collected during a ten-year period during viral suppression. To identify differentially expressed (DE) genes, surface protein markers, and pathway enrichment (PE) along the timeline of aging, we used the BD Rhapsody scRNA-seq ABseq platform. For cell typing, we performed ABseq using 30 antibodies to major diagnostic surface antigens of immune cells.

Results: The resulting integrated datasets consisted of >100,000 transcriptomes (>500 genes/cell) and ABseq profiles. We performed detailed DE gene and PE analyses in CD4 and CD8 T-cells, monocytes, NK, DC, and B-cells, and their sub-populations (CD4 naïve, TEM, TCM, and CTL). The most significant changes were between pre- and perimenopausal participants and less so between peri- and postmenopausal. Thus, TNFα signaling via NFκB, Interferon-α, p53, and complement showed significant enrichment in the cells of perimenopausal participants. Premenopausal CD4 T cells showed enriched PI3K/AKT/MTOR and androgen pathways, whereas genes of the late estrogen response, KRAS signaling, and glycolysis marked the postmenopausal stage (Figure 1). We observed similar DE genes and pathways in cross-sectional and longitudinal datasets.

Conclusion: Reproductive aging in WWH shifts transcriptome signatures in the immune cells toward innate immune pathways and antiviral cytotoxicity, possibly in response to the rise of inducible HIV reservoir. Future scRNAseq studies including matching males with HIV are necessary to further decipher sex-specific differences.

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Figure 1: Clustering of longitudinal cytokine trajectories of 26 cytokines into 4 clusters using kmShape to cluster cytokine trajectories based on shape using the Frechet distance. Y-axis is log-transformed and normalized cytokine concentration and x-axis is weeks since ART initiation. Bold colored lines are average value of all cytokines within a cluster. Thin colored lines are single cytokine trajectories with color indicating cluster membership.
We did not observe differences in RTL in total CD8 T cell between groups, than HNC (p< 0.01), but CD8 T-cell distribution was similar between groups. maturation subsets or T-regulatory cells were found. No differences were compared to HNC group (p< 0.01), while no major differences in the CD4 T-cell PLWH and HNC (median 3.6 IQR [2-5] and 3.25 IQR [2-5], respectively). Only there were no significant differences in the number of comorbidities between activated (HLA-DR+CD38+), senescent (CD57+, KRLG1+) and exhausted of CD4 and CD8 T cell subsets (CD45RA, CCR7, CD27, CD28 CD25, CD127), and of T cells with a telomere-specific peptide nucleic acid probe by spectral flow. Methods: from well clinically characterized older PLWH and HIV-negative individuals. We assessed markers of immunosenescence in CD4 and CD8 T cells present higher prevalence of ageing-associated morbidities than uninfected people living with HIV (PLWH) on antiretroviral therapy (ART) individuals independently of HIV serostatus.

264 DURABLE ART SUPPRESSION IN AGED RHESUS MACAQUES IS ASSOCIATED WITH MHC-I ALLELES

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Background: Aging is associated with impairments in immune function that greatly increase the risk of viral infection and disease progression. However, the effects of CD8+ T cell-restricted MHC-I haplotypes on viral dynamics in old age remain undefined. We investigated how MHC-I alleles associated with spontaneous control of viral replication impact primary SIVmac239 infection and viral suppression in plasma and central nervous system (CNS) with ART in aged rhesus macaques (RM).

Methods: 16 aged RM (17-23 years old), with markedly reduced naive T and B cells, were stratified into two groups based on expression of either Mamu A*01, B*08 or B*17 (protective MHC, n=8) or controls (no protective MHC, n=8) and IV inoculated with 2 IU of SIVmac239. At 70 days post-infection (dpi), a subset of 8 RM (n=4; protective MHC and n=4; controls) received daily ART consisting of dolutegravir, elvitegravir and tenofovir disoproxil (TDF) for 15 weeks followed by TDF monotherapy for a further 21 weeks. SIV loads in plasma (pvl) and cerebrospinal fluid (CSF) were assessed using real-time RT-qPCR.

Results: Upon SIV infection, pvl at peak, set point and total viral burden, as measured by area under curve (AUC) between 0 – 70 dpi were significantly lower in RM with protective MHC alleles relative to controls. Following ART, we observed a rapid decrease in pvl in RM with protective MHC, with all achieving pvl below threshold (15 RNA copies/ml) prior to TDF and this stringent viral suppression was maintained in plasma and CSF during TDF. In contrast, RM with no protective MHC alleles had significantly reduced viral suppression in response to ART and substantial viremia in plasma and CSF during TDF monotherapy.

Conclusion: Aged RM with CD8+ T cell-restricted MHC-I alleles associated with viral control demonstrated reduced SIV replication following primary infection and rapid suppression following ART that was maintained despite suboptimal therapy. Our results suggest that enhancing CD8+ T cell immunity may promote durable viral suppression, including in the CNS, in older HIV+ individuals.

265 GERMINAL CENTER B CELLS INDUCE TFH ACTIVATION AND HIV REPLICATION

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Background: T follicular helper cells (TFH) are major sources of HIV replication in people living with HIV infection. T cell activation enhances HIV replication, cytokine secretion including IL-2 and TNF, and expression of several receptors including OX40, CD25, CD69 and 4-1BB. In ex vivo infected tonsils, HIV replication is elevated in activated T cells expressing OX40 and reduced levels of CD127. Although much is known of the impact of TFH on germinal center B cell (GCB) activation and function, little is known of the influence of GCB on the activation state of TFH in HIV infection. We hypothesized that GCB induce activation and HIV replication in TFH.

Methods: TFH (7-AAD-CD3+CD8-CXCR5hiPD-1hi) and GCB (7-AAD-CD19+IgD-CD38+, CD27+, CD25+, CD69+) were sorted from tonsils of individuals at low risk of HIV infection undergoing routine tonsillectomies. TFH were spinoculated with X4- or R5-tropic GFP reporter virus and cultured for 3 days in the presence or absence of GCB in media with sucinilavir. Percent GFP+TFH and GFP MFI were measured by flow cytometry. In a subset of experiments, TFH were isolated after culture and RNA was extracted. Individual transcripts for 7/85 genes were quantified using an nCounter Host Response Panel and analyzed using Rosalind. Fold changes and p-values were calculated and p-value adjustment was performed using the Benjamini-Hochberg method of estimating false discovery rates.

Results: Compared to TFH alone, percent GFP+TFH and GFP MFI were elevated by a median of 11% and 28%, respectively, in X4-tropic infection when cultured with GCB (n=34; 12 female; p< 0.001, p< 0.001, respectively). Elevations in GFP+TFH (median, 37%) and GFP MFI (median, 48%) were also seen in R5-tropic...
SIALIDASE INHIBITION PREVENTS HIV-MEDIATED INFLAMMATION AND IMMUNE ACTIVATION IN VIVO

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**Background:** Sialylated glycoproteins (proteins containing the glycan sialic acid) are reduced during HIV infection (viremic and antiretroviral-therapy (ART)-suppressed), and levels of sialidase (enzyme cleaving sialic acid) are elevated. Sialylated glycoproteins initiate anti-inflammatory immune responses by engaging the inhibitory sialic-acid binding proteins (Siglecs) expressed on several immune cells. We tested whether inhibiting sialidase, via sialidase inhibitors commonly used clinically to treat influenza (oseltamivir and DANA), would attenuate HIV-mediated immune activation/inflammation in vivo.

**Methods:** Two independent cohorts of bone-marrow-liver-thymus (BLT) humanized mice were infected or not with HIV_SIVmac (a transmitter-founder virus) at 3,500 TCID50. Infected mice were treated (via daily oral gavage) with a combination of oseltamivir and DANA (25mg/kg; a clinically comparable dosage) or saline control. Normalizing glycosylation patterns (in particular sialylation) reduces levels of tissue-associated HIV DNA in humanized mice.

**Results:** Sialidase inhibition significantly attenuated HIV-mediated induction of several markers of inflammation and immune activation, including markers of the pro-inflammatory type-II interferon responses (p<0.05; Fig. 1A). In contrast, sialidase inhibition induced levels of the antiviral type-I interferon-α (p=0.06). Consistently, sialidase inhibition significantly attenuated HIV-mediated induction of CD8+ T cell activation and exhaustion in the blood (p<0.05; Fig. 1B) and tissues (p<0.05; Fig. 1C). This reduction in HIV-mediated inflammation and immune activation was accompanied with a decrease in plasma viral load (p=0.02; Fig. 1D) and levels of cell-associated HIV DNA in the liver, lungs, and spleen (p<0.02; Fig. 1E). The degree of immune activation and inflammation significantly correlated with higher levels of HIV viral load in plasma and tissues (p<0.05; Fig. 1G).

**Conclusion:** Normalizing glycosylation patterns (in particular sialylation) represents a novel approach to prevent the development of inflammation during HIV infection. Future studies are warranted to examine the potential impact of sialidase inhibition on HIV-mediated inflammation during suppressive ART.

**Figure 1:** Sialidase inhibitors attenuate immune activation/inflammation and reduce levels of tissue-associated HIV DNA in humanized mice.
268 IMPACT OF EARLY ART ON TISSUE RESIDENT MEMORY T CELLS IN THE GASTROINTESTINAL TRACT
Aloysius Ssemaganda1, Yuwadee Phuang-ngern2, Chadaya Sajaweerawan2, Phil Ehrenberg1, Henok Gebrebrihan1, Sandra Choi1, Michael Corley2, Giulia Severini1, Nittaya Phanuphak3, Carlo Sacdalan4, Denise Hsu5, Rasmi Thomas6 with antiretroviral therapy (ART). Based on our prior studies, we hypothesized that mucosal CD4 T cell frequencies more closely than by HIV status, with higher abundance in PWH who initiated ART early and PWOH (median 38.8% vs 47.2%, p< 0.0001), a 64% decrease in the frequency of total CD4 T cells was observed between PWH who initiated ART early and PWOH (median 38.8% vs 47.2%, p< 0.0001), a 64% decrease in the frequency of total CD4 T cells was observed between groups, suggestive of preferential decrease in CD4 Trm was observed between groups, suggestive of preferential depletion of CD4 Trm cells. Abs CD4 Trm, but not total CD4 T cells, correlated with mucosal CD4 T cell frequencies more closely than by HIV status, with higher abundance in PWH who initiated ART early and PWOH (median 38.8% vs 47.2%, p< 0.0001), a 64% decrease in the frequency of total CD4 T cells was observed between PWH who initiated ART early and PWOH (median 38.8% vs 47.2%, p< 0.0001), a 64% decrease in the frequency of total CD4 T cells was observed between PWH who initiated ART early and PWOH (median 38.8% vs 47.2%, p< 0.0001), a 64%

269 MULTIMOMIC BIOMARKERS ACROSS ART INITIATION TIMEPOINTS REVEAL INTERFEROME ENRICHMENT
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Background: People with HIV (PWH) have risk of comorbidities even before starting ART. Based on our prior studies, we hypothesized that persistent immune activation in PWH, particularly in the monocyte compartment, undermines immunoregulation in some PWH. We analyzed peripheral blood gene expression profiles of monocytes and lymphocytes from PWH before and after ART to assess regulation of the interferome in chronic immune dysfunction after ART.

Methods: We included cryopreserved PBMCs from PWH before starting ART (preART; with CD4 > 200 cells/mL, n=17) and < 1 year after starting ART (postART, n=17), as well as matched HIV-negative individuals (HN, n=12). RNAseq was performed on PBMCs and flow-sorted cells to identify longitudinal changes in gene expression. Samples were further subgrouped to early postART (sampled ≤60 days after ART initiation), and late postART (>100 days after ART initiation). Low input RNAseq was performed (Illumina NextSeq 500 platform) on flow-sorted cytometry-sorted cell subsets. Linear contrasts were performed to identify differentially expressed genes (DEG, p≤0.05, n=2) between preART, postART and HN. We employed single cell multiomic analysis (10X Genomics, ATAC) to delve deeper into possible mechanisms of epigenetic accessibility impacting gene expression in PWH before vs after ART administration.

Results: Overall, 237 interferon response genes (IRGs) in monocytes were differentially expressed in postART vs HN but not preART vs HN (p≤0.05). However, expression of gene sets representing IFN pathways and key regulators of immune responses, notch signaling, cell cycle, and intercellular communication were enriched in both postART and preART samples relative to HN (adj. p<0.05), indicating enrichment of these pathways during ART use. We also found significantly expressed IRG unique to monocyte subsets when comparing the postART versus earlyART PWH, with additional epigenetic molecular phenotypes distinguishing PWH endotypes before and after starting ART. Importantly, these molecular phenotypes persisted with ART.

Conclusion: These multiomic phenotypes represent candidate biomarkers and a growing understanding of the role monocytes play in immune dysfunction observed in some PWH endotypes with ART. Validation of these signatures in an independent cohort is needed to pursue these potential translational targets.
Antiviral and immunomodulatory therapies, and provide insights for the development of new therapies.

Methods: In plasma from individuals with COVID-19, sampled ≤10 days from symptom onset from the All-Ireland Infectious Diseases Cohort study, we measured 61 biomarkers, including markers of innate immunity and T cell activation, coagulation, tissue repair, lung injury, and immune regulation. We used principal component analysis (PCA) and k-means clustering to derive biomarker clusters, and univariate and multivariate ordinal logistic regression to explore association between cluster membership and maximal disease severity, adjusting for risk factors for severe COVID-19, including age, sex, ethnicity, BMI, hypertension and diabetes.

Results: From March 2020-April 2021, we included 312 individuals, (median (IQR) age 62 (48-77) years, 7 (4-9) days from symptom onset, 54% male) in the analysis. PCA and clustering derived 4 clusters. Compared to cluster 1, clusters 2-4 were significantly older and of higher BMI but there were no significant differences in sex or ethnicity. Cluster 1 had low levels of inflammation, cluster 2 had higher levels of markers of tissue repair and endothelial activation (EGF, VEGF, PDGF, TGFα, serpin E1 and P-selectin). Cluster 3 and 4 were both characterised by higher overall inflammation, but compared to cluster 4, cluster 3 had downregulation of growth factors, markers of endothelial activation, and immune regulation (IL10, PDG, STAT). In univariate analysis, compared to cluster 1, cluster 3 had the highest odds of severe disease (OR (95% CI) 9.02 (4.62-18.31), followed by cluster 4: 5.59 (2.75-11.72) then cluster 2: 4.5 (2.38-8.81), all p < 0.05). Cluster 3 remained most strongly associated with severe disease in fully adjusted analyses; cluster 3: OR (95% CI) 5.99 (2.69-13.35), cluster 2: 3.14 (1.54-6.42), cluster 4: 3.13 (1.36-7.19), all p < 0.05).

Conclusion: Distinct early inflammatory profiles predicted maximal disease severity independent of known risk factors for severe COVID-19. A cluster characterised by downregulation of growth factor and endothelial markers and early evidence of alveolar injury was associated with highest risk of developing severe COVID19. Whether this reflects a dysregulated inflammatory response that could improve targeted treatment requires further study.

Heatmap of biomarker derived clusters and forest plot of association between clusters and disease severity. A: Heatmap demonstrating differences in biomarker clusters, and univariate and multivariate ordinal logistic regression to explore association between cluster membership and maximal disease severity. A cluster characterized by higher overall inflammation, but compared to cluster 4, cluster 3 had downregulation of growth factors, markers of endothelial activation, and immune regulation (IL10, PDG, STAT). In univariate analysis, compared to cluster 1, clusters 2 and 3 had higher odds of severe disease (OR (95% CI) 9.02 (4.62-18.31), followed by cluster 4: 5.59 (2.75-11.72) then cluster 2: 4.5 (2.38-8.81), all p < 0.05). Cluster 3 remained most strongly associated with severe disease in fully adjusted analyses; cluster 3: OR (95% CI) 5.99 (2.69-13.35), cluster 2: 3.14 (1.54-6.42), cluster 4: 3.13 (1.36-7.19), all p < 0.05).

Conclusion: Distinct early inflammatory profiles predicted maximal disease severity independent of known risk factors for severe COVID-19. A cluster characterized by downregulation of growth factor and endothelial markers and early evidence of alveolar injury was associated with highest risk of developing severe COVID19. Whether this reflects a dysregulated inflammatory response that could improve targeted treatment requires further study.

Heatmap of biomarker derived clusters and forest plot of association between clusters and disease severity. A: Heatmap demonstrating differences in biomarker clusters between B: Forest plot demonstrating odds ratio of specific clusters for progressing to moderate or severe disease (reference Cluster 1), calculated using ordinal logistic regression. Odds ratio (95% CI) presented as unadjusted and fully adjusted (for age, sex, ethnicity, BMI, hypertension, diabetes, immunosuppression, smoking and baseline anticoagulant use).

Maximal disease severity graded per the WHO severity scale.

Figure 1. Lipid profiles from COVID-19 susceptible and non-susceptible individuals obtained by LC-MS at positive and negative ionisation modes. A: OPLS-DA score plot of LC-MS (ESI) (R2 = 0.98; Q2 = 0.99). B: ANOVA. C: Heatmap of common significant lipids obtained by LC-MS for susceptible and non-susceptible groups.

The X-axis order on the right represents the relative abundance. Samples for susceptible individuals are depicted in red. Samples for non-susceptible individuals are depicted in green. Samples for non-susceptible individuals are depicted in red in real.

272 EARLY INFLAMMATORY PROFILES PREDICT MAXIMAL DISEASE SEVERITY IN COVID-19

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Background: Better understanding of host inflammatory changes that precede development of severe COVID-19 could improve delivery of available

patterns also indicate a change in fatty acid metabolism. HIV viremia may impact innate function through metabolic and epigenetic regulatory pathways.

271 PLASMA LIPIDS AND AMINO ACIDS LINKED TO LOW SARS-CoV-2 SUSCEPTIBILITY

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Background: The mechanisms driving SARS-CoV-2 susceptibility remain poorly understood, especially the factors determining why a subset of unvaccinated individuals remain unaffected despite high-risk exposures.

Methods: We studied an exceptional group of unvaccinated healthcare workers heavily exposed to SARS-CoV-2 (‘non-susceptible’) from April to June 2020, who were compared against ‘susceptible’ individuals to SARS-CoV-2, including uninfected subjects who became infected during the follow-up, and hospitalized patients with different disease severity providing samples at early disease stages. We analyzed plasma samples using different mass spectrometry technique and obtained metabolites and lipids profiles.

Results: We found that the metabolite profiles were predictive of the selected study groups and identified lipids profiles and metabolites linked to SARS-CoV-2 susceptibility and COVID-19 severity. More importantly, we showed that non-susceptible individuals exhibited unique metabolomics and lipidomic patterns characterized by upregulation of most lipids —especially ceramides and sphingomyelin—and amino acids related to tricarboxylic acid cycle and mitochondrial metabolism, which could be interpreted as markers of low susceptibility to SARS-CoV-2 infection. Lipids and metabolites pathways analysis revealed that metabolites related to energy production, mitochondrial and tissue dysfunction, and lipids involved in membrane structure and virus infectivity were key markers of SARS-CoV-2 susceptibility.

Conclusion: Lipid and metabolic profiles differ in ‘non-susceptible’ compared to individuals susceptible to SARS-CoV-2. Our study suggests that lipid profiles are relevant actors during SARS-CoV-2 pathogenesis and highlight certain lipids relevant to understand SARS-CoV-2 pathogenesis.

Lipid profiles from COVID19 patients obtained by LC-MS

A

B

C

Figure 1. Lipid profiles from COVID19 susceptible and non-susceptible individuals obtained by LC-MS at positive and negative ionisation modes. A: OPLS-DA score plot of LC-MS (ESI) (R2 = 0.98; Q2 = 0.99). B: ANOVA. C: Heatmap of common significant lipids obtained by LC-MS for susceptible and non-susceptible groups.

The X-axis order on the right represents the relative abundance. Samples for susceptible individuals are depicted in red. Samples for non-susceptible individuals are depicted in green. Samples for non-susceptible individuals are depicted in red in real.
DIFFERENTIAL ASSOCIATION OF CYTOMEGALOVIRUS WITH ACUTE AND POST-ACUTE COVID-19

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IMPACC, COMET, and LLNRC study teams
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Background: Asymptomatic Cytomegalovirus (CMV) infection reshapes systemic immune responses and its replication can be both a consequence and cause of inflammation. As CMV resides in the same tissues affected by SARS-CoV-2, we hypothesized that asymptomatic CMV co-infection might modify the pathogenesis of both acute and post-acute COVID-19.

Methods: Participants had current or prior nucleic acid-confirmed SARS-CoV-2 infection in the COVID-19 Multi-Phenotyping for Effective Therapies (COMET, n=219), Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC, n=244) or the Long-term Impact of Infection with Novel Coronavirus (LLNRC, n=327) cohorts. We assessed the relationship between CMV serostatus and odds of hospitalization and plasma SARS-CoV-2 N antigen levels during acute COVID-19 as well as post-acute “Long COVID” symptoms, defined as >=1 of 32 COVID-19-attributed symptoms present at least 60 days following initial symptom onset.

Results: Among 758 participants, 518 were hospitalized for their acute COVID-19 episode. CMV seropositivity was independently associated with a 1.9-fold increased odds of hospitalization for acute COVID-19, after adjustment for age, sex, race, ethnicity, HIV status, prior autoimmune disease, diabetes, and obesity (p=0.01, A). Among those hospitalized, CMV seropositivity was also associated with higher plasma SARS-CoV-2 N antigen levels (median 936 vs. 323 pg/ml, P=0.03, B), which remained significant after adjustment for potential confounders, but not with ICU admission (n=209), death (n=58), or thrombotic events (n=31). In contrast to its relationship to acute COVID-19 disease severity, CMV seropositivity was independently associated with a 48% decreased odds of having neurocognitive Long COVID symptoms such as headache and brain fog 4 months after initial COVID-19 diagnosis (P=0.036). Conversely, serologic evidence of Epstein-Barr Virus (EBV) reactivation and HIV both increased the odds of these symptoms (C).

Conclusion: CMV seropositivity is associated with a 1.9-fold higher odds of hospitalization in people with acute COVID-19 and a nearly 3-fold higher SARS-CoV-2 antigen burden in hospitalized patients. In contrast, CMV seropositivity is associated with a decreased odds of neurocognitive Long COVID symptoms, while other chronic viral co-infections like EBV reactivation and HIV are associated with an increased odds of this complication. The biological mechanisms mediating these relationships are unknown, but warrant further investigation.

Association of demographic and disease factors with acute and post-acute COVID-19 outcomes.

BASELINE AND TEMPORAL DIFFERENCES IN PROTEOMICS FOLLOWING SARS-CoV-2 INFECTION IN PWH

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Background: COVID-19 may be more severe in persons with HIV (PWH). However, underlying biological mechanisms associated with the development of COVID-19 and its clinical severity among antiretroviral therapy (ART) treated PWH are largely unknown. Therefore, we wished to evaluate temporal changes in plasma proteins following SARS-CoV-2 infection and identify pre-infection proteomic markers associated with future COVID-19.

Methods: We analyzed the data of clinical, antibody-confirmed COVID-19 ART-treated PWH from the global Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE). Individuals were matched on geographic region, age, and sample timing to antibody-negative controls. For cases and controls, pre-COVID-19 pandemic specimens were obtained prior to January 2020 to assess temporal changes and baseline differences in protein expression in relationship to COVID-19 severity, using mixed effects models adjusted for false-discovery rate.

Results: We compared 257 unique plasma proteins (Olink Proteomics) in 94 COVID-19 antibody-confirmed clinical cases and 113 matched antibody-negative controls, excluding COVID-19 vaccinated participants (median age 50 years, 73% male). 40% of cases were characterized as mild; 60% moderate to severe. Median time from COVID-19 infection to follow-up sampling was 4 months. Temporal changes in protein expression differed based on COVID-19 disease severity. Among moderate to severe cases vs. controls, NOS3 increased, whereas TSG6 decreased. Higher baseline circulating concentrations of granzymes A, B and H (GZMA, GZMB and GZMHB) were associated with the future development of moderate-severe COVID-19 in PWH and were related to immune function, including CD4, CD8 and the CD4/CD8 ratio.

Conclusion: We identified temporal changes in novel proteins in closely linked inflammatory, immune, and fibrotic pathways which may relate to COVID-19-related morbidity among ART-treated PWH. Further, we identified key granzyme proteins, serine proteases expressed by cytotoxic T lymphocytes and NK cells in response to foreign antigens, associated with future COVID-19 in PWH. Our results provide unique insights into the biological susceptibility and responses to COVID-19 infection in PWH.

Baseline and temporal expression differences following SARS-CoV-2 infection in ART-treated PWH

Baseline and temporal expression differences following SARS-CoV-2 infection in ART-treated PWH

SARS-CoV-2 INFECTION IN PWH

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Gladstone Institutes, San Francisco, CA, USA

Background: The continuous evolution of SARS-CoV-2 in the diverse immune landscape (natural, vaccine, hybrid) is giving rise to novel immune escape mutations. So far, the resulting new variants (BA.1, BA.2, BA.2.12.1) were observed to cause mild infections, however, BA.5 infections are associated with
an increased risk of hospitalization. Therefore it is essential to investigate the pathogenesis of BA.5.

**Methods:** Here we compared the pathogenicity of Pre-Omicron (B.1.351) and Omicron (BA.1, BA.2.1.12.1, and BA.5) variants in wild-type C57BL/6 mice and K18-hACE2 mice. The virus replication kinetics was also studied in human Calu3, pulmonary alveolar type 2 (AT2) cells, and airway organoids (HAO). Cell-to-cell spread of virus was measured by syncytia formation assay and immunohistochemistry (IHC) of infected lungs.

**Results:** In the results, infection in C57BL/6 mice showed severe weight loss (>15%) for B.1.351 infected mice and moderate (~5%) for BA.5 infected. C57BL/6 mice showed higher virus replication of B.1.351 followed by BA.5, BA.1, and BA.2.12.1. At the peak of virus replication (2 days) plaque-forming units from lung extract of BA.5 infected mice were two, and three logs higher compared to BA.1 and BA.2.12.1 respectively. BA.5 infection was lethal to 80% of infected K18-hACE2 mice, whereas the mice looked normal after infection with BA.1 and BA.2.12.1. BA.5 infected mice showed high virus replication in brain tissue. Surprisingly the syncytia formation assay and IHC for BA.5 was comparable to that of B.1.351, indicating the higher cell-to-cell spread of BA.5 and B.1.351 compared to BA.1 and BA.2.12.1, which is one of the measures of pathogenicity. Calu3 and HAO showed the same trend of virus replication as was observed in-vivo experiments however AT2 cells were found to be resistant to BA.5 replication.

**Conclusion:** These results suggest that the BA.5 variant (lineage) of Omicron has the potential to regain the pathogenicity as it shows increased virulence compared to other Omicron sub-variants. Lethal infection of BA.5 in K18-hACE2 mice may be attributed to catastrophic encephalitis and increased cell-to-cell spread.

### 276 AUTOPHAGY AND ACYL-COA-BINDING PROTEIN INFLUENCE COVID-19 SEVERITY

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**BQC-19 Biobank**

Research Institute of McGill University Health Centre, Montréal, QC, Canada, Research Institute of McGill University Health Centre, Montréal, Montreal, QC, Canada, Centre de recherche des Cordeliers, Paris, France, McGill University Health Centre, Montréal, QC, Canada

**Background:** Autophagy, a cytosolic structure degradation pathway, allows production of IL21 by CD4 T-cells and efficient cytokine responses by CD8 T-cells. Autophagy is in part regulated by acyl-CoA-binding protein (ACBP) which has two functions. Intracellular ACBP favors autophagy, whereas secreted ACBP is related to extracellular factor inhibition and increased cell-to-cell spread.

**Methods:** To examine early inflammatory patterns in people with (Ob) and without (NOb) obesity and COVID-19 and how they relate to COVID-19 disease severity.

**Results:** Of 100 subjects (50 Ob and 50 NOb) presenting between April 2020 and March 2021, characteristics (Ob vs NOb) included: age 65 [23-91] vs 65 [21-95], female sex 27 [48%] vs 28 [56%], BMI 33.7 [30.0-73.8] vs 33.3 [15.3-32.9], disease severity mild 22 [48%] vs 23 [46%], moderate 15 [30%] vs 13 [26%], severe 6 [12%] vs 7 [14%], HTN 30 [60%] vs 17 [34%], DM 19 [38%] vs 6 [12%], days from symptom onset 7 [2-17] vs 8 [1-15], vaccinated 3 [6%] vs 0 [0%]. Compared to NOb, Ob had higher IFNα (1.8 [0.6; 11] vs 0.9 [10; 4.7]), CRP (10 [10-30]/µL [9/3; 10.2] vs 9.7 [7.2-10]), IL-1ra (197 [132; 299] vs 138 [88-253]), IL-1B (8.4 [6.2; 17] vs 7.8 [6.6-12.9]), IL-6 (3482 [1513; 5738] vs 848 [249; 2114]). Plasma ACBP levels correlated with LC3II levels (r=0.27, p<0.001), IL21 (r=0.51, P<0.001) and IL6 (r=0.41, p<0.001), but neither with markers IL1β (8.4 [6.2; 17] vs 7.8 [6.6-12.9]), IL-6 stimulation of healthy control PBMC induced extracellular ACBP release. Moreover, adding recombinant ACBP: 1) reduced autophagy in lymphocytes and monocytes upon polyclonal stimulation with PMA/ionomycin or LPS; 2) reduced intracellular production of IL21 in T-cells after PMA/ionomycin stimulation.

**Conclusion:** Plasma ACBP levels were inversely linked with IL21 levels, suggesting that autophagy and IL21 allow control of SARS-CoV-2 infection, independently of the level of SARS-CoV-2 antibody secretion. ACBP is a targetable autophagy checkpoint and its extracellular inhibition may improve SARS-CoV-2 immune control.

### Table 1: Demographic and results summary

<table>
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<th>Fatal</th>
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### 277 INFLAMMATORY PATHWAYS ASSOCIATED WITH COVID-19 SEVERITY DIFFER BASED ON OBESITY STATUS

**Stefano Savinelli**, Grace Kenny, Colm O’Brien, Kathleen McCann, Alejandro Garcia Leon, Gurvin Saioti, Colette Marie Gaillard, Rya Negri, Dana Alalwan, Alan Landay, Cecilia O’Kane, Aofie Cotter, Patrick Mallon, Eoin R. Feeney

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**Background:** Severe COVID-19 and obesity are characterized by higher inflammation. We aimed to examine early inflammatory patterns in people with (Ob) and without (NOb) obesity and COVID-19 and how they relate to COVID-19 disease severity.

**Methods:** Ob (BMI >30 kg/m²) and NOb with COVID-19 matched for age, sex and WHO disease severity provided blood early after diagnosis. Immunoassays measured 57 plasma biomarkers reflecting innate immune and endothelial activation, systemic inflammation, coagulation, metabolism and microbial translocation (Fig 1). Between-group differences were assessed by Mann-Whitney. Associations between subsequent maximal COVID-19 severity (mild vs moderate/severe/critical) and biomarkers were explored by logistic regression adjusted for age, sex, hypertension (HTN) and diabetes (DM). Data are median pg/mL [IQR] or n (%) unless stated.

**Results:** Out of 100 subjects (50 Ob and 50 NOb) presenting between April 2020 and March 2021, characteristics (Ob vs NOb) included: age 65 [23-91] vs 65 [21-95], female sex 27 [48%] vs 28 [56%], BMI 33.7 [30.0-71.8] vs 33.3 [15.3-32.9], disease severity mild 22 [48%] vs 23 [46%], moderate 15 [30%] vs 13 [26%], severe 6 [12%] vs 7 [14%], HTN 30 [60%] vs 17 [34%], DM 19 [38%] vs 6 [12%], days from symptom onset 7 [2-17] vs 8 [1-15], vaccinated 3 [6%] vs 0 [0%]. Compared to NOb, Ob had higher IFNα (1.8 [0.6; 11] vs 0.9 [10; 4.7]), CRP (10 [10-30]/µL [9/3; 10.2] vs 9.7 [7.2-10]), IL-1ra (197 [132; 299] vs 138 [88-253]), IL-1B (8.4 [6.2; 17] vs 7.8 [6.6-12.9]), IL-6 (3482 [1513; 5738] vs 848 [249; 2114]), and lower S100B (318 [231; 398] vs 350 [233; 491]), IL-1α (8.4 vs 7.2 [4.7; 10]), IL-1β (57 [30; 97] vs 68 [30-119]), Zonulin (114 ng/mL [77; 131] vs 57 [18; 106]), Resistin (956 [539; 1153] vs 318 [231; 398]), IL-1RA (197 [122; 399] vs 138 [88-253]). In both groups higher, proinflammatory IL-1β and lower levels of anti-inflammatory CCL22 and IL-5 were associated with higher odds of disease severity, and lower E-selectin with higher disease severity only in Ob. However, in NOb higher type 3 interferons (IL-28A), macrophage activation (SCD163, CCL3) and vascular inflammation markers (ICAM-1, VCAM-1), along with higher ST208, GM-CSF and leptin were also associated with disease severity, a pattern not observed in Ob (Fig 1).

**Conclusion:** Although Ob had higher overall levels of inflammation than NOb, few biomarkers predicted subsequent COVID-19 severity in Ob. These differential inflammatory patterns suggest dysregulated immune responses in Ob with COVID-19.
278 SARS-CoV-2 VARIANTS-DEPENDENT INFLAMMASOME ACTIVATION
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Background: Innate immunity is the first line of defense in response to pathogens, which acts locally and also leads the stimulation of adaptive immunity through at least with IL-1β secretion. It has been shown that SARS-CoV-2 infection triggered the NLRP-3 inflammasome activation and the IL-1β secretion. The aim of this study was to analyze and compare the level of IL-1β secretion that is one of the most important innate immunity cytokines, in monocyte-like cells infected with 6 different variants of the SARS-CoV-2.

Methods: Six SARS-CoV-2 variants (historical (B.1, D614G), Alpha, Beta, Gamma, Delta and Omicron BA.1) were isolated from COVID-19 hospitalized patients. Viral stocks were obtained by inoculation in Vero and Vero-TRMPSS2 cells. THP-1 monocyte-like cells were cultured with RPMI-hepes 10% FBS-0.05% penicillin/streptomycin. Cells were treated with LPS 1µg/ml for 2h and infected with different SARS-CoV-2 variants with a MOI 0.1. IL-1β secretion was measured by luminex assay in the supernatant after 24 h of infection.

Results: We analyzed and compared IL-1β secretion between SARS-CoV-2 virus 6 sublineages after infection of monocytes like THP-1. We observed that THP-1 cells infected with SARS-CoV-2 variants presented a significantly higher IL-1β secretion than non-infected cells. Moreover, some SARS-CoV-2 variants led to a stronger IL-1β secretion, and particularly we observed a significantly higher level of IL-1β cells infected with Omicron BA.1 sublineage compared to other variants.

Conclusion: We observed the inflammasome activation for the 6 SARS-CoV-2 sublineages with a variation in level of IL-1β secretion. Indeed, our results suggested that Omicron BA.1 was more recognized by the innate immune cells than other SARS-CoV-2, which could in part, with its upper respiratory tract tropism, possibly explain its less clinical virulence. Taking together, these results suggest that the innate immune response and precisely, IL-1β secretion pathways were activated in a SARS-CoV-2 variants-dependent manner.
RBD sufficiently similar to ACE2 to yield ACE2-like catalytic activity – ACE2-like ‘abzymes’.

**Methods:** To explore this hypothesis, we studied 67 patients hospitalized with COVID-19 who had disodium ethylenediaminetetraacetate (EDTA) anticoagulated plasma samples available, obtained about 7 days after admission. We used commercially available fluorometric ACE2 assays (Abcam), and a SpectraMax® M5 microplate reader (Molecular Devices), measuring Relative Fluorescent Unit (RFU, Ex/Em = 320/420 nm; RFU) in a kinetic mode every 20 min at 37°C. ACE2 inhibitor provided in the assay kit was used for additional controls. In some control experiments, we added Zn2+ to plasma, or conducted serial dilutions to decrease Zn2+. To deplete Igs, we passed plasma samples through a 0.45 µm filter to remove large particles, then passed the material through 100kDa cut-off ultrafiltration membrane (Pierce™) columns, and finally used protein A/G Magnetic Beads (Life Technologies) to specifically material through 100kDa cut-off ultrafiltration membrane (Pierce™) columns, and finally used protein A/G Magnetic Beads (Life Technologies) to specifically deplete Ig, removing >99.99% of Ig as assessed with a human IgG ELISA Kit (Abcam).

**Results:** ACE2 is a metalloprotease that requires Zn2+ for activity. However, we found that the plasma of 11 of the 67 patients could cleave a synthetic ACE2 peptide substrate, even though the plasma samples were collected using EDTA anticoagulant. When we spiked plasma with synthetic ACE2, no ACE2 substrate cleavage activity was observed unless Zn2+ was added, or the plasma was diluted to decrease EDTA concentration. After processing samples by size exclusion and protein A/G adsorption, the plasma samples did not cleave the ACE2 substrate peptide.

**Conclusion:** The data suggest that some patients with COVID-19 develop Igs with activity capable of cleaving synthetic ACE2 substrate. Since abzymes can exhibit promiscuous substrate specificities compared to the enzyme whose active site image they resemble, and since proteolytic cascades regulate physiologic processes, anti-RBD abzymes may contribute to some otherwise obscure features of COVID-19 pathogenesis. Hypothesized Anti-RBD Antibodies with Catalytic Activity: Anti-RBD Abzymes

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**SARS-CoV-2 Variants’ Temporal and VL Distributions in Immunocompromised Patients**

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ANRS MIE – EMERGEN Consortium

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**Background:** The COVID-19 pandemic has been striking for three years and, despite the regular arise of new variants, populations are now widely immune and protected from severe symptoms. However, immunocompromised patients still have worse clinical outcomes, higher mortality and rarely develop effective immunity through vaccination or infection. Here, we studied the temporal distribution of infections, viral loads (VL) as well as the viral genetic diversity among an immunocompromised patient cohort, between January 2021 and September 2022.

**Methods:** Overall, 478 immunocompromised patients (solid organ transplant, HIV positive, cancer, autoimmune disease) and 234 controls (healthcare workers) from Pitité-Salpêtrière and Bichat Claude-Bernard University hospitals (Paris, FRANCE) were diagnosed with SARS-CoV-2 infection by RT-qPCR. Whole genome sequencing was performed according to ARTIC protocol on Oxford Nanopore platform. All 712 full viral genomes were used to determine lineages and mapped to Wuhan-Hu-1 reference to produce a maximum likelihood phylogenetic tree (IQTree, 1000 bootstraps). Differences in temporal distributions of infections and VL were assessed using nonparametric statistical tests.

**Results:** According to phylogenetic analysis, genomes from SARS-CoV-2 infecting immunocompromised patients and those infecting healthy individuals are distributed in a similar way. No significant genetic differences can be observed between viral genomes from patients and controls within the different lineages. Temporal distribution of COVID-19 infections were also similar between immunocompromised patients and controls, with the exception of BA.2 variant for which controls were infected earlier \(p<0.001\). VL were significantly lower in immunocompromised patients infected with Omicron variants \(p=0.04\). No differences in VL were observed for Alpha and Delta variants.

**Conclusion:** At diagnosis, no intrinsic genetic divergence was observed in virus infecting immunocompromised patients compared to those circulating in the general population. Similarities in temporal distribution of infections between controls and patients suggest that these different groups become infected concomitantly. VL appeared to be lower for Omicron variants in immunocompromised patients. An earlier VL peak of Omicron and a testing of immunocompromised patients hospitalized once severe symptoms have appeared could indicate a delayed testing in these patients, once the replicative phase over.

SAR-CoV-2 viral loads in immunocompromised patients vs controls

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**PLASMA-BASED ANTIGEN PERSISTENCE IN THE POST-ACUTE PHASE OF SARS-CoV-2 INFECTION**

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**Background:** There is mounting evidence regarding the frequency and spectrum of post-acute sequelae of SARS-CoV-2 infection (PASC), but a search for causes has been elusive. Recently, a plasma-based assay for SARS-CoV-2 antigen has been developed, which in initial use revealed that a high fraction of severely affected patients with PASC had circulating antigen. It is unknown whether detectable SARS-CoV-2 antigen is specific for PASC or how the assay performs in a broader clinical spectrum of patients with PASC.

**Methods:** We evaluated a cohort of patients with RNA-confirmed SARS-CoV-2 infection enrolled ≥3 weeks following initial symptoms. Participants, both with and without PASC at enrollment, were identified via facility- and community-based advertising and examined every 4 months. An interviewer-administered questionnaire ascertained presence of 30 different symptoms (new or worse compared to pre-COVID) in the prior 2 days at each exam. Using the single molecule array (Simoa) assay, we measured spike, S1, and nucleocapsid SARS-CoV-2 antigens in plasma collected at time of symptom assessment.

**Results:** We examined 172 participants (50% men, 46% non-white, median age 46 years) who contributed 667 timepoints from 0.7 to 15.4 months following infection, at which 66% featured report of ≥1 symptom. Sixty-one of 667 timepoints (9.1%) representing 24% of persons had ≥1 detectable SARS-CoV-2 antigen. Among the 437 timepoints at which ≥1 symptom was present, 9.8% had ≥1 detectable antigen; this compares to 7.8% of timepoints at which symptoms were absent. In comparison to those without symptoms, individuals with several specific symptom complexes (gastrointestinal, musculoskeletal, and central neurologic) more commonly had detectable antigen (Figure). Hospitalization during acute COVID-19 was strongly related to antigen detection.
Conclusion: Among a diverse group of SARS-CoV-2-infected persons in the post-acute phase of infection, SARS-CoV-2 antigen is detectable in plasma in both those with and without symptoms but more commonly in those with gastrointestinal, musculoskeletal, and central neurologic complaints. The findings indicate that antigen persists in at least some persons and suggest (but do not prove) that antigen is causally related to symptoms. That antigen is found in only a fraction of those with PASC indicates either that not all symptoms are driven by antigen, current plasma antigen detection is insensitive relative to tissue, or nominal PASC symptoms are sometimes unrelated to SARS-CoV-2.

Associations between SARS-CoV-2 antigen and COVID-attributed symptoms during the post-acute phase

283 PLASMA ANTIBODY AND N ANTIGEN STATUS PREDICT OUTCOMES IN OUTPATIENTS WITH COVID-19

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≥2500 pg/ml (Figure). Compared to individuals who had N Ag levels < LLoQ at risk of hospitalization or death, ranging from 1% for < 3 pg/ml to 70% for ≥2500 pg/ml. Participants with higher N Ag levels at day 0 had an increased risk of hospitalization or death, ranging from 1% for < 3 pg/ml to 70% for ≥2500 pg/ml. Participants with anti-Spike levels < LLoQ compared to those who had quantifiable anti-Spike at day 0, had an increased risk of hospitalization/death (16% vs 2%, RR [95% CI]: 0.8 [0.6, 1.0]). The overall median positivity duration was 15[95% CI: 13-16] days in the first and 2nd and 8[95% CI: 7-10] in the third wave (p= 0.007). Positivity duration was significantly higher in males (16 versus 14 days, p=0.03) and people aged >40 years (15 versus 14 days, p=0.02). Positivity duration was not affected significantly by age (p=0.05) and sex (p=0.03). The overall median positivity duration was 15[95% CI: 13-16] days with 15[95% CI: 13-16] in the first, 17[95% CI: 14-20] in the second and 8[95% CI: 7-10] in the third wave (p= 0.007). Positivity duration was significantly higher in males (16 versus 14 days, p=0.03) and people aged >40 years (15 versus 14 days, p=0.02). Positivity duration was not affected by presence or absence of symptoms (p=0.80). No significant correlation was found with viral load (p=0.03; p=0.61). Considering baseline (24±7±2dC) and last viral load (29.3±5.9 Ct), the ΔCt (4.6±1.3) and positivity duration (15 days) revealed a kinetic in viral decay of 0.3±0.087 Ct/day.

Conclusion: A median positivity duration of 15 days is in accordance with viral clearance around 2 weeks for optimal confinement at community-level. Men and/or the elderly stand at higher risk of prolonged infection. Given the viral decay (0.3 Ct/day), we suggest personalized confinement periods. The variability of positivity duration according to phases could be function of strains which could be a factor of positivity duration.

284 VIRAL DYNAMICS AND FACTORS FAVOURING THE DURATION OF COVID-19 POSITIVITY IN CAMEROON

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Background: Reliable biomarkers of COVID-19 severity and outcomes are critically needed for clinical and research applications. We evaluated associations between anti-Spike IgG and SARS-COV-2 nucleocapsid antigen (N Ag) in plasma with clinical outcomes in outpatients with COVID-19.

Methods: We conducted a prospective cohort-study of SARS-CoV-2 positive cases from the first to third wave (March 2020-October 2021) in Yaounde-Cameroon. RT-PCR was performed on nasopharyngeal swabs. SARS-CoV-2 positivity duration was evaluated from the first to last positive test before a negative result. Epi-info V.7.0 was used for data analyses with p< 0.05 considered statistically significant.

Results: A total of 282 participants were enrolled. The mean age was 41±14 years, with male predominant (62.1%). We had 15.6% symptomatic cases and cough most common (59.09%). The overall median positivity duration was 15 days (IQR: 9-23) days with 15 days (IQR: 13-16) in the first, 17 days (IQR: 11-26) in the second and 8 days (IQR: 4-12) in the third wave (p= 0.007). Positivity duration was significantly higher in males (16 versus 14 days, p=0.03) and people aged >40 years (15 versus 14 days, p=0.02). Positivity duration was not affected by presence or absence of symptoms (p=0.80). No significant correlation was found with viral load (p=0.03; p=0.61). Considering baseline (24±7±2dC) and last viral load (29.3±5.9 Ct), the ΔCt (4.6±1.3) and positivity duration (15 days) revealed a kinetic in viral decay of 0.3±0.087 Ct/day.

Conclusion: A median positivity duration of 15 days is in accordance with viral clearance around 2 weeks for optimal confinement at community-level. Men and/or the elderly stand at higher risk of prolonged infection. Given the viral decay (0.3 Ct/day), we suggest personalized confinement periods. The variability of positivity duration according to phases could be function of strains which could be a factor of positivity duration.
MITOCHONDRIAL DYSFUNCTION IS ASSOCIATED WITH POST-ACUTE SEQUELAE OF COVID-19

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Background: Mitochondrial (mt) dysfunction has been described in acute severe SARS-CoV2 infection. It remains unclear whether the disturbances in mt function are also present in post-acute sequelae of COVID-19 (PASC).

Methods: We analyzed cross-sectional data from participants without history of COVID and SARS-CoV2 antibody negative (COVID-), with documented prior COVID and full recovery (COVID+PASC+), and with prior COVID with PASC as defined by the CDC (COVID+ PASC-). Mt respiration was measured from peripheral blood mononuclear cells utilizing the Seahorse XF96 analyzer. Generalized linear regression was used to compare estimates of mt and non-mt respirations, and unadjusted odds ratios using multinomial logistic regression to assess if mt respiration were associated with PASC.

Results: For this analysis, 59 participants were enrolled, 71.9% (n=42) had a confirmed COVID-19 diagnosis. The overall mean age was 47.47 ± 14.86 years, 69.49% (n=41) were females and 33.90% (n=20) were non-white race. There was no difference in demographics between participants with and without COVID (p=0.72). Amongst all COVID+ participants, 19% (n=11) had hypertension and 8% (n=5) had diabetes. Among all COVID+, the median time between COVID diagnosis and study evaluation was 210 (IQR: 119, 453) days, and 50% (n=21) of COVID+ experienced persistent symptoms consistent with PASC. PASC participants had the highest observed values in non-mt respiration (21.57 ± 10.77 pmol/min), basal respiration (38.95 ± 17.58 pmol/min), proton leak (10.41 ± 3.1), and ATP production (28.55 ± 14.83 pmol/min). Basal respiration, ATP production, maximal respiration, and ATP production increased the predicted odds of PASC by 10.99, 5.6, 1.6 and 6.2%, respectively (Figure).

Conclusion: Individuals with PASC are consuming more oxygen and producing more ATP in the PBMCs compared to controls. There also appears to be increased PBMC ATP production between PASC and COVID+. We hypothesize that this may reflect a crucial pathogenic mechanism in PASC that may be associated with ongoing inflammation.

287 ADAPTIVE IMMUNITY DYSREGULATION IS ASSOCIATED WITH THE DEVELOPMENT OF LONG COVID

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Background: The pathogenetic mechanisms behind the development of long-COVID (LC) are largely unknown. Because both plasma SARS-CoV-2 RNAemia and dysregulated immunity have been correlated with COVID-19 severity, we evaluated whether they are associated with LC.

Methods: We consecutively enrolled unvaccinated hospitalized COVID-19 patients during acute-COVID-19 (T0) in March-October 2020 who either developed LC at a follow-up visit ≥2 months from virologic clearance (T1) or did not. LC was defined as persistence ≥2 months after recovery of ≥1 symptom: anosmia, dysgeusia, fever, gastrointestinal symptoms, dyspnea, fatigue, musculoskeletal pain, muscle weakness, brain fog. We measured: SARS-CoV-2 RNAemia (RT-qPCR, log10 copies/mL), magnitude (ELISA, AUC) and functionality (pseudovirus neutralization, IgG, IgM mediated functions, %ADCC) of SARS-CoV-2-specific antibodies, SARS-CoV-2-specific B and CD4-T cells (immunophenotype, AIM and ICS assays).
Results: We enrolled 48 COVID-19 individuals, 38/48 (79.2%) developed LC (LC+) and 10 did not (LC-). LC+ and LC- had similar co-morbidities and symptoms in the acute phase (Fig.1A), and the majority showed a radiologically documented SARS-CoV-2 pneumonia. The SARS-CoV-2 RNAemia did not differ between groups at both time points. The levels of RBD-specific Abs, as well as their functionality, appeared to increase over time in the LC- group but not in the LC+ (Fig.1B-D). Similarly, a trend towards increased RBD-specific B-cells was observed over time in the LC- group but not in LC+ (Fig.1E). B-cell immunophenotyping showed a significant increase over time of classical memory B cells (MBCs) at the expenses of activated MBCs (Fig.1F-G) as well as an IgA class-switching in the LC- group compared to LC+ (Fig.1H-I). Furthermore, LC+ showed a faster decline of SARS-CoV-2-specific (CD69 + CD137 +) CD4 TEMRA and CD4-TEM (Fig.1L-M). Finally, IFN-γ-producing TREG of LC- individuals increased over time (Fig.1N).

Conclusion: Acutely ill, hospitalized COVID-19 patients developing LC feature a dysregulated SARS-CoV-2-specific humoral as well as B- and T-cell response, in both magnitude and functionality, suggesting a link between dysregulated SARS-CoV-2-specific adaptive immunity and LC development. The fine understanding of the factors contributing to such dysregulation in LC patients is strongly needed, that might further inform targeted therapeutic interventions.

Methods: A cross-sectional study including COVID+ with PASC, COVID+ without PASC, and COVID- participants. We measured plasma markers by enzyme-linked immunosorbent assay to assess gut-barrier integrity: zonulin for intestinal permeability, lipopolysaccharide-binding protein (LBP) for microbial translocation, and fatty acid binding protein I-FABP for intestinal integrity, and high-sensitivity C-reactive protein (hsCRP) and oxidized low-density lipoprotein (Ox-LDL) assays.

Results: 415 participants were enrolled in our study. 62.17% (n=258) were without PASC, and COVID- participants. We measured plasma markers by enzyme-linked immunosorbent assay to assess gut-barrier integrity: zonulin for intestinal permeability, lipopolysaccharide-binding protein (LBP) for microbial translocation, and fatty acid binding protein I-FABP for intestinal integrity, and high-sensitivity C-reactive protein (hsCRP) and oxidized low-density lipoprotein (Ox-LDL) assays.

Conclusion: PASC is associated with increased gut permeability, which in turn is associated with oxidized LDL and hsCRP.

Zonulin (ng/mL) and Oxidized Low-Density Lipoprotein (U/L) by COVID-19 and PASC status

289 NONHUMAN PRIMATE MODEL OF LONG COVID: IMMUNE INSIGHTS OF GLYCOMETABOLIC DYSFUNCTIONS

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Background: A major consequence of COVID-19 is long-term metabolic complications (metabolic PASC or Long COVID) following acute disease resolution leading to hyperglycemia, increased risk of diabetes or defects in glucose metabolism. However, the mechanisms underlying the links between COVID-19 and glycometabolic disruptions remain unclear.

Methods: 15 African green monkeys (AGM; Chlorocebus aethiops) were infected with SARS-CoV-2 (Wuhan strain) and divided into two groups: unvaccinated (n=10) and vaccinated (BNT162b2) (Pfizer) 4-days post infection; n=5). Subgenomic SARS-CoV-2 mRNA (sgRNA) reflecting active replication was quantified in nasal and pharyngeal swabs, and blood chemistry analysis was performed longitudinally up to 18 weeks post-infection. We quantified liver glycogen at necropsy using Periodic acid–Schiff staining. Finally, we longitudinally analyzed 96 plasma proteins using a proximity extension assay (Olink). STRING was used to identify enriched protein networks. Comparisons between the two groups over time were performed using PERMANOVA.

Results: All animals had detectable sgRNA (>3.64x10^6) at day 3, and only two were undetectable at week 5. Post-infection BNT162b2 vaccination partially inhibited the SARS-CoV-2 mediated disruption of glucose levels (P=0.001, Fig. 1A). Liver glycogen levels following necropsy correlated positively with blood glucose levels at week 12 (r=0.74, P=0.003). Histopathological analysis revealed no marked evidence of long-term inflammation or fibrosis of pancreatic islets. Using the plasma proteomic data, we identified a signature of 15 SARS-CoV-2-modulated plasma proteins coinciding with early onset hyperglycemia during acute infection (P=0.001, Fig. 1B). These proteins are enriched for biological processes linked to chemotaxis (FDR=1.38E-06), and viral protein interaction with cytokines (FDR=1.01E-12) (Fig. 1C). Of these, CCL25 and glial cell derived neurotrophic factor (GDNF) remained persistently elevated post-acute infection and correlated with blood glucose levels (r=0.57, P=0.0001; and r=0.64, P<0.0001, respectively, Fig. 1D).

Conclusion: Our AGM model validates phenotypes of metabolic PASC and offers an opportunity to mechanistically study the manifestations of PASC. Our preliminary data suggest that vaccine-preventable early insults by metabolic-regulating immune factors may contribute to long-term dysregulated liver
and systemic glucose homeostasis during PASC. These immune factors warrant further investigation for their mechanistic links to PASC. SARS-CoV-2 infection of AGM is associated with early-onset hyperglycemia that persists.

290 PROLONGED SARS-CoV-2 VIRAL BURDEN IN SIV-SARS-CoV-2 COINFECTED MACAQUES
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Background: Individuals living with HIV are at increased risk of morbidity and mortality from COVID-19. Furthermore, SARS-CoV-2 infection in immunocompromised HIV infected individuals poses a risk to prolonged infection and viral shedding and the emergence of new variants of concern (VOCs). Using the SIV macaque model for AIDS, we are investigating the hypothesis that immune dysfunction during HIV infection will prolong SARS-CoV-2 viral infection, promote enhanced COVID-19 disease, and accelerate viral evolution. Here, we report the impact of SIV-CoV-2 co-infection on immune responses and pathogenesis.

Methods: Eight female rhesus macaques (aged 7-15 years, 5.5-9.9kg) were infected with SIVmac251 via low dose intravaginal challenge and then inoculated with 6.5x10^5 TCID50/mL SARS-CoV-2 (WA-1) at 17-34 weeks post-SIV infection via combined intranasal and intratracheal routes. Blood, bronchoalveolar lavage (BAL), stool, and nasal, oral, and rectal swabs were collected pre-infection through 14 days post-infection (DPI) to measure immune responses and viremia. ELISAs, ELISPOT, qRT-PCR, lung pathology, cytokine multiplex, and virus neutralization assays were performed to measure viral loads, pathogenesis, and immune responses.

Results: Three days post-SARS-CoV-2 infection, we observed a transient decrease in CD4 counts, but there were no changes in clinical symptoms or plasma SIV viral loads. However, SARS-CoV-2 replication persisted in the upper respiratory tract, but not the lower respiratory tract. In addition, SARS-CoV-2 IgG seroconversion was delayed and antigen-specific T-cell responses were dampened. Notably, viral RNA levels in nasal swabs were significantly higher 7-14 DPI in SIV+ compared to previously published results using the same SARS-CoV-2 challenge virus in SIV-rhesus (PMCID: PMC8462335, PMC829873). In addition, SIV/CoV-2 co-infected animals exhibited elevated levels of myeloperoxidase (MPO), a marker of neutrophil activation and increased lung inflammation.

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 immunosuppression during SIV infection impairs de novo generation of anti-SARS-CoV-2 immunity, that may 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 if SARS-CoV-2 viral evolution is accelerated in SIV-infected macaques.

291 DELAYED POSITIVIZATION OF NON-LESION SPECIMENS AMONG INDIVIDUALS WITH MPXV
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Background: Viral dynamics of non-lesion mpox specimens is uncertain, with possible community implications regarding mpox transmission. The aim was to investigate the delayed positivization of non-lesion specimens among individuals diagnosed with mpox.

Methods: We included individuals diagnosed with mpox at San Raffaele Scientific Institute, Milan; all individuals had mpox positive lesions at time of mpox diagnosis. Delayed positivization was defined as mpox detection at virological re-evaluation in a non-lesion specimen which was previously negative at time of mpox diagnosis; plasma (Pl), urines (Ur), seminal fluids (Sf), oropharyngeal (Op) and anal (An) swabs were collected among all people. Individuals were virologically re-assessed until mpox clearance.

Results: Overall, 1836 total samples (1246 non-lesion specimens; 478 positive and 768 negative) from 140 MSM diagnosed with mpox were considered. Viral dynamics of non-lesion specimens negative at time of mpox diagnosis are illustrated in Figure 1. Observed delayed positivizations were 24 (11 Op, 5 Pl, 4 Ur, 3 Sf, 1 An) among 10% of included individuals. Among them, the median time to mpox diagnosis from symptoms onset was 2 days (interquartile, IQR 1-3, range 1-5); all were already in care at our center for HIV/PEP and half were sexual contacts of mpox cases. Median cycle thresholds (Ct) of samples at time of delayed positivization were: Op 31 (IQR 26-33), Pl 34 (IQR 33-36.5), Ur 33 (IQR 32-35), Sf 36 (IQR 35-37), and An 36. Median days to positivization from mpox diagnosis were: Op 7 (IQR 7-9), Pl 7 (IQR 3.5-10), Ur 7 (IQR 6.5-8), Sf 9 (IQR 8-10), and An 7. In Op, Ur, Sf, and samples with delayed positivization, plasma was already positive at time of mpox diagnosis (median Ct 34, IQR 33-35). The median Ct of mpox lesions at time of mpox diagnosis was 24 (IQR 19-28), at time of delayed positivization 20.5 (IQR 17-25); among all individuals appearance of new mpox lesions was documented following mpox diagnosis.

Conclusion: Delayed positivization can occur in non-lesion specimens among individuals diagnosed with mpox; high Ct values, positivization at first virological re-evaluation and more often at the oropharyngeal site were observed. We hypothesize that individuals received a prompt mpox testing and that the delayed positivization followed bloodstream viral dissemination. Given the community implications on transmission, these data reinforce the need of repeated mpox testing of non-lesion specimens.

Figure 1. Mpox testing dynamics at virological re-evaluation of non-lesion specimens which tested negative at mpox diagnosis; all individuals had mpox positive lesions at time of mpox diagnosis. Left: Non-lesion specimens with delayed positivization, plasma was already positive at time of mpox diagnosis (median Ct 34, IQR 33-35). The median Ct of mpox lesions at time of mpox diagnosis was 24 (IQR 19-28), at time of delayed positivization 20.5 (IQR 17-25); among all individuals appearance of new mpox lesions was documented following mpox diagnosis.

Positive
Negative
LONGITUDINAL ASSESSMENT OF VIRAL SHEDDING AMONG PATIENTS WITH MPOX IN TORONTO, CANADA

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Background: Patients with human Mpox infection may have detectable virus in multiple anatomic compartments including skin, mucosal sites, blood, urine and semen. Longitudinal data on viral shedding are scarce.

Methods: We conducted a prospective cohort study of adults with confirmed or suspected Mpox infection at an academic hospital in Toronto, Canada. Participants underwent swabs of skin lesions (up to three anatomic sites per visit), the nasopharynx (NP), the oropharynx, and the rectum, as well as urine and semen sampling, regardless of symptom status, at weekly (+/-3 days) visits until one week after complete resolution of the last skin lesion. Refrigerated specimens were transported to the laboratory within 24 hours of collection, frozen at -80°C, and batch tested for Mpox virus using quantitative polymerase chain reaction (qPCR) targeting the viral polymerase gene E9L. We report serial cycle threshold (CT) values for specimens taken from participants with confirmed infection.

Results: Between 21JUN2022-12OCT2022, we enrolled 28 participants, all of whom were cisgender men who have sex with men. Median (range) age was 39 (29, 60) years, and 12/28 (43%) were HIV-positive, all but one of whom had an undetectable viral load at the time of enrollment. Clinical manifestations ever reported included skin lesions (100%), headache (75%), fatigue (68%), fever (64%), myalgias (54%), rectal pain/discharge (46%) and sore throat (39%). After excluding specimens collected during or after use of tecovirimat, 21 participants (64%), myalgias (54%), rectal pain/discharge (46%) and sore throat (39%).

Conclusion: Mpox virus genetic material may remain detectable in multiple anatomic compartments for up to 8 weeks after symptom onset. Correlation with infectivity requires further study.

INNATE IMMUNITY IN HTLV-1 INFECTION: IFN-ГAMMA LEVELS ARE UP-REGULATED IN EARLY HAM

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Background: HTLV-1 infection is responsible for two aggressive diseases, leukemia and neuronal inflammation (HAM), in 5-10% of the chronically-infected asymptomatic carriers (AC). However, some infected individuals present minor symptoms that do not meet the criteria for HAM, while not asymptomatic which we named Intermediate syndrome (IS). To determine whether immune responses could discriminate IS from AC and healthy carriers (HC), and could be used as biomarkers of disease progression, in this study, were analyzed the frequency of monocytes (cMono, intMono, and ncMono), dendritic cells (DCs), and NK cells and their cytokine production, before and after stimulation.

Methods: 23 HTLV-1-positive participants being followed up at the Institute of Infectious Diseases “Emilio Ribas” were invited to participate in this study. Whole fresh blood samples were collected from healthy controls (HC) (n=8), asymptomatic carriers (n=8), and individuals with the intermediate syndrome (n=15), and in vitro stimulated with R848, an agonist of TLR7/8 to evaluate the responsiveness of immune cells. Multi-parameter immunostaining was contact among mpox cases during the 21-days monitoring period, regardless of their symptoms. However, it is unknown whether seminal fluids might represent a viral reservoir. Therefore, we aimed at evaluating mpox DNA clearance in semen samples of people with mpox infection over six months of follow-up.

Methods: This is an observational, prospective, single-center study of outpatients diagnosed with mpox in IRCSS San Raffaele, Milan, Italy between May, 2022, and October, 2022. Samples were analyzed by a real-time polymerase chain reaction (RT-PCR) to detect the presence of non-variola DNA; a specific RT-PCR targeting mpox DNA was used for confirmation. The primary endpoint was time from the first positive test of seminal fluid (baseline, BL) to viral DNA clearance. Follow-up accrued from BL until the date of most recent test of seminal fluid (performed within 6 months).

Results: Overall, 164 seminal fluid samples were collected from 140 people diagnosed with mpox infection; mpox DNA was detected in seminal fluid of 43 people with a BL median cycle thresholds of 34 (IQR 31-36). At mpox diagnosis, median age was 36 (IQR 34-42) years, 96% were men who had sex with men (MSM), 23% had concomitant sexually transmitted infections (7 C. trachomatis, 4 N. gonorrhoeae, 2 syphilis) and 28% were living with HIV infection. To assess viral clearance in seminal fluid, 58 further seminal samples were collected in 32 individuals (11 people has only BL sample). Viral clearance was observed within 6 months in all these individuals in a median time of 10.5 days (IQR 7-33); 19/28 seminal samples tested within 1 week were negative (68%), 25/28 (89%) tested negative within 2 weeks, 26/29 (90%) within 3 months (Figure 1).

Conclusion: These preliminary findings from this cohort of individuals highlight that viral DNA clearance in seminal fluid samples from people diagnosed with mpox infection was mostly observed within 2 weeks since first positive test. These findings suggest that semen testing and prolonged use of condoms after mpox infection may be necessary.

Figure 1 - Time to viral clearance in study individuals

INNATE IMMUNITY IN HTLV-1 INFECTION: IFN-ГAMMA LEVELS ARE UP-REGULATED IN EARLY HAM

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Background: HTLV-1 infection is responsible for two aggressive diseases, leukemia and neuronal inflammation (HAM), in 5-10% of the chronically-infected asymptomatic carriers (AC). However, some infected individuals present minor symptoms that do not meet the criteria for HAM, while not asymptomatic which we named Intermediate syndrome (IS). To determine whether immune responses could discriminate IS from AC and healthy carriers (HC), and could be used as biomarkers of disease progression, in this study, were analyzed the frequency of monocytes (cMono, intMono, and ncMono), dendritic cells (DCs), and NK cells and their cytokine production, before and after stimulation.

Methods: 23 HTLV-1-positive participants being followed up at the Institute of Infectious Diseases “Emilio Ribas” were invited to participate in this study. Whole fresh blood samples were collected from healthy controls (HC) (n=8), asymptomatic carriers (n=8), and individuals with the intermediate syndrome (n=15), and in vitro stimulated with R848, an agonist of TLR7/8 to evaluate the responsiveness of immune cells. Multi-parameter immunostaining was
performed to analyze the cytokine production of different cell subpopulations, here specifically the IFN-α production of pDC. In addition, we quantified the spontaneous IFN-γ production by peripheral blood mononuclear cells (PBMCs) using ELISPOT assay. One-way ANOVA or Kruskal-Wallis statistical analysis was performed using the software GraphPad Prism 8.0 to compare parametric or nonparametric variables.

**Results:** 31 volunteers were included (77% women and 23% men), with a median age of 56 years. No statistical difference was seen in the cell subpopulations of the three groups. However, IFN-α production by pDC cells (fig. 1a) was significantly reduced in the IS vs HC group (p=0.002). Moreover, spontaneous IFN-γ production (fig. 1d) was increased in both asymptomatic and IS individuals compared to HC, with a statistical difference of p=0.0013 between HC and AC and of p=0.0011 between HC and IS.

**Conclusion:** Changes in innate and adaptive immunity are observed in HTLV-1 infected individuals compared to healthy donors regardless of their disease status. A higher decrease of IFN-α-producing pDC and a higher increase of IFN-γ-producing PBMCs in IS compared to AC might be a signature of disease evolution to HAM.

Main changes observed in the immune response of this study

**Graphical abstract

**HIV coinfection increases HBV-induced hepatic fibrogenesis through HIF-1α**

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**Background:** The progression of chronic HBV to cirrhosis is accelerated by HIV coinfection compared to HBV mono-infection. HIV and gp120 signal through CCR5 and CXCR4 on hepatocytes and hepatic stellate cells to promote cell growth and proliferation through hypoxia-inducible factor-1α (HIF-1α). HIF-1α has been shown to regulate expression of the pro-fibrogenic cytokine TGF-β1.

We hypothesize that HIV and gp120 promote HBV-induced liver fibrosis in HIV/ HBV coinfected cell culture models through HIF-1α signaling.

**Methods:** Infectious HBV viral particles (HBVvp) were purified from the HBV supernatant (HBVsup) of HepAD38 cells. HIV NL4-3 strains were propagated in U937 macrophage lines and directly incubated with LX-2 stellate cells and hepatoma HepG2 and HBV-infected NTCP-HepG2 cells. Cells were incubated with recombinant HIV proteins including Gp120, Tat, Vpr, Vif, Nef, Ref, Vpu, reverse transcriptase, protease, and integrase. HBV subgenomic constructs including S, Core, X, and P were transfected into NTCP HepG2 cells. CXCR4 (plerixafor) and CCR5 (maraviroc) inhibitors were used to block HIV-induced liver fibrosis.

**Results:** HIV infection significantly increased expression of HIF-1α and TGFB1 in U937 cells in a virus load-dependent manner. HIF-1α and pro-fibrogenic genes were also significantly upregulated in HBV-infected NTCP HepG2 cells compared to uninfected cells. HIV-infected U937 cell supernatant and recombinant HIV gp120 significantly increased expression of HIF-1α, TGFB1 and profibrotic genes in HBV-infected NTCP-HepG2 and LX2 cells. The overexpression of HBV X protein significantly upregulated pro-fibrogenic gene expression and this can be enhanced by the exposure of HIV and gp120 peptide. HIF-1α siRNA transfection and CCR5/CXCR4 inhibitors neutralized the HIV- and HIV gp120-induced fibrogenic response.

**Conclusion:** HIV and HBV coinfection exacerbates liver fibrogenesis through upregulation of the HIF-1α pathway. This cooperative effect can be mitigated by disruption of the interaction between HIV/Gp120 and its coreceptors CCR5/ CXCR4.
ileal mucosa of children with CD compared to healthy children and its correlation to the expression of genes involved in mucosal inflammation.

**Methods:** We used online available high throughput RNA-sequencing data of ileal biopsies of 26 children with CD and of 8 age-matched healthy controls. We used Bowtie2 for the alignment of these data against the hg19 human genome assembly; we used Samtools and Bedtools to retrieve read counts corresponding to 13 HERV families (HERV-H, HERV-W, HERV-L, HERV-E, HERV-I, HERV-9, HERV-FRD, HML-1/2/3/4/5/6) and a panel of 20 mucosal immunity genes. We evaluated HERV expression at a family level by summing the reads corresponding to each HERV family. HERV and immunity gene expression was normalized by means of four housekeeping genes (SDHA, HPRT1, RBX1, RRAGA). Normalized expressions were In-transformed and we used independent sample t-tests, to compare the expression of HERV families and immunity genes in the ileal biopsies of children with CD and controls. Spearman’s rho correlation coefficient was used to identify correlations between the expression of differentially expressed HERVs and immunity genes.

**Results:** We recognized upregulated expression of HERV-FRD (1.54-fold, \( p = 0.031 \)) and downregulated expression of HERV-9 (0.52-fold, \( p = 0.013 \)) in children with CD. Of the examined immunity genes, we found significant upregulation in the expression of CXCL-10 (5.9-fold, \( p = 0.001 \)), CXCL-11 (4.3-fold, \( p = 0.001 \)), CXCL-16 (1.64-fold, \( p = 0.002 \)), SPANK (1.4-fold, \( p = 0.014 \)), ADAM-10 (1.5-fold, \( p = 0.047 \)), TLR-4 (2.1-fold, \( p = 0.036 \)) in CD. HERV-FRD expression correlated to the expression of SPANK (Rho=0.68, \( p = 0.001 \)) and ADAM-10 (Rho=0.411, \( p = 0.037 \)), while HERV-9 expression correlated to the expression of CXCL-10 (Rho=-0.496, \( p = 0.001 \)), CXCL-11 (Rho=-0.5, \( p = 0.009 \)) and TLR-4 (Rho=-0.5, \( p = 0.008 \)).

**Conclusion:** We recognized dysregulated expression of HERV-FRD and HERV-9 in children with CD, correlated to the expression of genes involved in proinflammatory processes in ileal mucosa and the perpetuation of mucosal inflammation. These findings merit further research on the HERV role in intestinal mucosa fitness to confirm the HERV involvement in pediatric IBD.

**MYCOBACTERIUM TUBERCULOSIS ASSOCIATES WITH HIGHER HIV-1-SPECIFIC ANTIBODY RESPONSES**

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**Background:** Mycobacterium tuberculosis (Mt) can enhance immune responses against unrelated pathogens. Mt is the most common co-infection in people living with HIV (PW), and the impact of active Mt disease on HIV-1 immune responses has not been examined.

**Methods:** Neutralization and antibody dependent cellular cytotoxicity (ADCC) were compared among plasma samples from PW and Mt disease (PW/Active Mt) and PWN/No Mt both prior to any treatment and after antiretroviral treatment (ART) and completion of Mt therapy. Single genome amplification, flow cytometry and luminescencing assays were used to examine HIV-1 envelope sequences, antibody, and cytokine levels respectively. Wilcoxon-rank sum, Kruskal Wallis, and Student t-tests were used to compare different groups and multivariate linear regression analysis was used to account for baseline demographic differences.

**Results:** HIV-1 neutralizing antibodies (nAbs) were broader and more potent in PW/Active Mt (n = 15) as compared to PWN/No Mt (n = 37, \( p < 0.0001 \)) even after accounting for pre-treatment plasma virus level and CD4 counts. NAb breadth and potency significantly increased among the PW who developed active Mt after ART initiation (PW/Active Mt + ART, \( n = 9 \)) as compared to the PWN/No Mt + ART (\( n = 10 \)) and PWN/No Mt + ART (\( n = 22, p = 0.011 \)). ADCC was higher in PW/Active Mt + ART as compared to PWN/Active Mt + ART and PWN/No Mt + ART (\( p = 0.02 \)), but ADCC was not different among PW/Active Mt and PWN/No Mt prior to ART initiation. Before ART, PW/Active Mt as compared to PWN/No Mt had unique HIV-1 envelope sequence motifs associated with neutralization resistance implying differences in humoral immune selection pressure. The Mt-linked antibody augmentation associated with elevated plasma levels of interleukin-6, a proliferation-inducing ligand (APRIL), and B-cell activating factor (BAFF), but not other cytokines important for B cell development and maintenance. Active Mt co-infection did not associate with non-specific elevation of all antibodies or cross-reactive responses.

**Conclusion:** Mt disease enhances HIV-1 antibody responses primarily as a bystander effect on pathways important for antibody production. The Mt enhanced humoral responses do not associate with increased virus levels or envelope antigen diversity. Understanding pathways perturbed by Mt disease can provide unique insights for inducing optimal HIV-1 antibody responses. Neutralization breadth and potency prior to (left) and post ART initiation (right)

**IMPACT OF THE INTRAUTERINE HIV EXPOSURE ON THE INFANTS VIRUS ANTIBODY REPERTOIRE**

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**Background:** Antibody responses to vaccines do not explain health outcomes disparities observed between infants who are HIV-exposed but uninfected (iHEU) and HIV-unexposed uninfected (iHUU). We investigated the effect of HIV-exposure on infant humoral immunity by comparing viral-specific antibody repertoires between iHEU and iHUU.

**Methods:** IgG against 206 eukaryote-infecting viruses was measured using Phage Immunoprecipitation Sequencing in plasma of iHEU (\( n = 15 \)) and iHUU (\( n = 15 \)) at birth and 36 weeks of life. Data analysis was performed using AntiViral Antibody Response Deconvolution Algorithm (ARVADA). High-dimensional reduction analysis and pairwise comparison was performed in R with alpha < 0.05 as the significance level.

**Results:** We measured viral specific antibodies from 30 study participants at birth and 36 weeks of life, 42.85% of whom were female. High-dimensional reduction analysis revealed age-associated difference in the repertoire of virus-targeting antibodies between paired birth and week 36 samples (\( p < 0.001 \)). At birth, antibody repertoires were different between iHEU and iHUU (\( p = 0.002 \)), although they converged by week 36 (\( p = 0.790 \)). iHUU had a greater number of unique viral antibody targets per participant at birth than iHEU (median; 9 vs. 6; \( p = 0.035 \)), although there were no differences by week 36 (median; 7 vs. 6; \( p = 0.500 \)). Antibodies against HIV were detected in 10 iHEU at birth and at week 36, respectively, showing a >2-fold longitudinal decrease (\( p < 0.001 \)). Common viral targets at birth included Epstein-Barr virus (100%), herpes simplex-1 (90.0%) and -2 (73.3%), cytomegalovirus (93.3%), mastadenovirus C (63.3%) and rhinovirus A (96.6%), whereas antibodies against enterovirus B (70.0%) and C (70.0%), cytomegalovirus (76.6%), rhinovirus A (83.0%) and B (60.0%) were common at week 36. Mastadenovirus C antibody levels were higher in iHUU compared to iHEU at birth (mean; 28.06 vs. 24.27; \( p = 0.048 \)).

**Conclusion:** Lower antibody repertoire breadth of iHEU at birth could have clinical significance including increased risk of infections, warranting further investigations. Further analysis of the maternal antibody repertoire is required to determine whether the differences observed between iHEU and iHUU at birth are as result of impeded transplacental antibody transfer or different antibody targeting antibodies between paired birth and week 36 samples (\( p < 0.001 \)). At birth, antibody repertoires were different between iHEU and iHUU (\( p = 0.002 \)), although they converged by week 36 (\( p = 0.790 \)). iHUU had a greater number of unique viral antibody targets per participant at birth than iHEU (median; 9 vs. 6; \( p = 0.035 \)), although there were no differences by week 36 (median; 7 vs. 6; \( p = 0.500 \)). Antibodies against HIV were detected in 10 iHEU at birth and at week 36, respectively, showing a >2-fold longitudinal decrease (\( p < 0.001 \)). Common viral targets at birth included Epstein-Barr virus (100%), herpes simplex-1 (90.0%) and -2 (73.3%), cytomegalovirus (93.3%), mastadenovirus C (63.3%) and rhinovirus A (96.6%), whereas antibodies against enterovirus B (70.0%) and C (70.0%), cytomegalovirus (76.6%), rhinovirus A (83.0%) and B (60.0%) were common at week 36. Mastadenovirus C antibody levels were higher in iHUU compared to iHEU at birth (mean; 28.06 vs. 24.27; \( p = 0.048 \)).
Background: Dissecting broadly neutralizing antibody (bnAb) specificity in polyclonal plasma in HIV-1 infection and post vaccination remains challenging. Delineation methods comparing plasma neutralization patterns against large virus panels with those of epitope-mapped bnAbs are widely used. Common to the current approaches is their inability to resolve the presence of multiple bnAb specificities, or, in rare occasions, of novel bnAb types. Here we report on virus panel classification, a novel delineation method designed to overcome these limitations and probe its utility on the XbnAb cohort, a large cohort of bnAb inducers (N=304) identified in the Swiss 4.5K Screen (Ruset et al. 2016).

Methods: Plasma neutralization fingerprints of bnAb inducers (N=304) were evaluated using a 40-virus multiclade panel and compared to reference fingerprints of bnAbs (V1V2, V3 glycans, CD4bs, interface, fusion peptide, MPER) and broadly neutralizing DARPin mAbs (bnD) (Friedrich et al. 2021). bnAb specificity was predicted using a maximum Spearman correlation method and the newly developed "virus panel classification" strategy. Creating thousands of random possible panels (size 10 to 35) from an existing data set, this method computationally identifies 20 to 30 virus sub-panels with optimal ability to classify bnAbs into their respective epitope clusters. Plasma specificity is predicted on each virus sub-panel using maximum Spearman correlation, plasma clusters based on the percentages of prediction for each epitope in all virus panels and specificities defined based on the observed signatures in each plasma cluster.

Results: Compared to the classical Spearman approach, we find agreement in 58% of plasmas with the panel method including only bnAbs as reference fingerprints, and 91% of plasmas when additionally integrating bnDs. The virus panel classification allowed a more detailed allocation of bnAb specificity and fingerprints, and 91% of plasmas when additionally integrating bnDs. The virus panels and specificities defined based on the observed signatures in each plasma cluster.

Conclusion: Application of the new viral panel classification to the XbnAb cohort provided a highly reliable prediction of bnAb specificity. In addition, the method provides insight into potential multi-specific bnAbs responses, indicating its usefulness for analyzing bnAbs responses in future vaccine trials.

301 INFERRING IMMUNOGENIC PROPERTIES OF ENV TRIMERS BY REACTIVITY TO bnAb INDUCER PLASMA
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Background: Identification of HIV-1 envelope (Env) trimers with the ability to induce broadly neutralizing antibodies (bnAbs) remains critical for HIV-1 vaccine development. Here, we used the XbnAb cohort, comprising bnAb inducers (N=304), and non-neutralizing (nnAb) controls (N=304) identified in the Swiss 4.5k study (Ruset Nat Med 2016), to investigate the reactivity of 29 candidate Env trimers with polyclonal bnAbs and nnAb responses in HIV-1 infection. Several of these trimers immunogens have been clinically tested or are in the testing phase, including BG505 SOSIP, BG505 DS-SOSIP, ConM SOSIP, V7, BG505 SOSIP.v4 1-GT1.1 and ConCV5 KIKO. Together with the wealth of available data on the XbnAb cohort, including information on the induced bnAb type, virus, disease parameters, and host demographics, this provided a unique framework to obtain information on the in vivo immunogenic potential of these Env trimers.

Methods: Patient plasma were assessed for IgG1, -2, and -3 against 29 stabilized soluble Env trimers in a Luminesa-based binding antibody multi-plex assay (BAMA). Plasma dilution curves were summarized into weighted MFI. We determined the quality of bnAbs engaging trimers immunogens using the following criteria: i) MFI magnitude in bnAb inducers, ii) differentiation between bnAb inducers and nnAb controls, iii) reactivity with different subtype plasma, iv) reactivity with plasma with different predicted bnAb type.

Results: We first ranked Env trimers for their level of reactivity and capacity to differentiate between bnAb and nnAb inducers based on IgG1. Trimers that stood out in this were BG505 SOSIP.v4 1-GT1.1 (and derivatives), ConM SOSIP.v7, and ConCV5 (and derivatives). Subtype reactivity (comparison of plasma from subtype B versus non-B infection) was not significantly different in the majority of Env when adjusting for ethnicity, and the few exceptions were modest in magnitude, confirming that antigenic features on the Env trimers are mostly conserved across subtypes. Stratifying plasma based on the bnAb type elicited provided insights into differential binding preferences of Envs trimers, that are in part intended as in the case of germline targeting immunogens.

Conclusion: Weighting the antigens according to their ability to discriminate bnAb from nnAb inducers, as well as an in-depth analysis of their reactivity patterns, provided unique clues to their immunogenic potential that can be exploited for vaccine development.

302 GLYCAN-DEFICIENT SHIVS TO ELICIT BROADLY NEUTRALIZING ANTIBODIES IN MACAQUES
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Background: Previous studies have shown that HIV-1 SOSIP Env trimers or cleavage-independent native flexibly linked trimers engineered to lack glycans surrounding the CD4 binding site (CD4bs) or Fusion Peptide (FP) can immunofocus potent NAb responses to these critical domains. However, such immunogens did not elicit neutralization breadth, possibly due to that Env epitopes could not co-evolve and sustain sufficient affinity gradients to select for antibody affinity maturation. Here, we designed novel replicating simian-human immunodeficiency viruses (SHIVs) to encode primary transmitted/received Env that lacked glycans at residues surrounding the CD4bs (197, 363, and 462) or the FP (88, 241, and 611) to test the hypothesis that native-like SHIV Env could selectively prime, boost and affinity-mature neutralizing or broadly neutralizing antibodies (bnAbs) targeting these key epitopes.

Methods: We introduced mutations disrupting glycosylation sequences at the above residues to generate eight glycan-deficient SHIV designs in previously characterized transmitted/received SHIVs. We infected a cohort of 16 Indian-origin rhesus macaques intravenously with one of eight designs and followed them longitudinally to evaluate viral kinetics, Env sequence evolution, and neutralizing antibody development.

Results: 11 of 16 animals exhibited ideal viral kinetics and were used for further analysis. 10 of 11 animals developed potent antigenic neutralizing responses targeting peptides beneath the engineered glycan holes. 8 of 11 animals developed responses capable of neutralizing heterogeneous glycan-deficient viral strains. Longitudinal single genome sequencing revealed a rapid, sequential restoration of the deleted glycans, which enabled virus escape from neutralization. In a subset of animals, additional mutations known to affect CD4bs or FP bnAbs arose concomitantly with rising neutralizing titers. 4 macaques developed antibody responses capable of neutralizing wild-type heterogeneous viruses: 3 targeting the CD4bs, and 1 targeting the FP.

Conclusion: These results demonstrate a highly dynamic process of Env-Ab coevolution leading to viral resistance and maturation of immunofocused B cell responses. A subset of animals developed CD4bs or FP targeted bnAbs. Ongoing work will continue to monitor these animals’ neutralizing responses and isolate monoclonal antibodies from animals with wild-type heterogeneous neutralization. These studies will help inform the design of boosting immunogens to elicit CD4bs or FP bnAbs.

303 IDENTIFICATION OF HIV BROADLY NEUTRALIZING MONOCLONAL ANTIBODIES TARGETING MPER
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Background: Broadly neutralizing monoclonal antibodies (bnAbs) target sites of vulnerability on the HIV envelope, such as the gp41 membrane proximal external region (MPER). MPER-directed bnAbs, such as VRC01 and PGZ11, have been previously isolated from chronically infected CCR5_01 AE and subtype B infected donors, respectively, and are part of the 4E10-class of MPER-specific bnAbs. Here we report the isolation of MN01P01 from a subtype B infected donor, as another 4E10-class bnAb. These results indicate that MPER-directed mAbs are found in multiple donors infected with different HIV subtypes.
Methods: Monoclonal antibodies (mAbs) were isolated from a subtype B infected donor using two parallel methods: high-throughput B-cell culture followed by microneutralization assays, and fluorescently labeled MPR peptide to capture antigen-specific B cells by cell sorting. B-cell receptors were sequenced, and heavy and light chain sequences were cloned into expression vectors and expressed in Expi293F cells. mAbs were then characterized for binding, epitope mapping, and neutralization across a panel of 208 diverse pseudoviruses (pSVs). mAbs with potent cross-neutralization were further characterized by crystal structure determination.

Results: MPR-specific mAbs were isolated using both MPR-specific B-cells and probe negative B-cells, and confirmed by neutralization using an HIV-2 chimera pSV containing HIV-2 backbone with HIV-1 MPR inserted. Two mAbs, MHRP.01 and MHRP.02 were able to neutralize 100% and 97% of the pSVs in a panel of 208, respectively with an average potency ≤ 1μg/mL. MHRP.01 utilized VH1-69 and VK3-20, the same gene usage as other MPR-specific mAbs VRC42, and PGZL.1. MHRP.01-GW was produced to include shared amino acid residues with VRC42, glycine (G) and tryptophan (W), and exhibited more robust binding to the MPR peptide but did not improve neutralization compared to MHRP.01. Crystallization of MHRP.01 revealed residues important for MPR binding, which bound in the same conformation as MPR-directed nAbs 4E10 and VRC42 (Fig. 1).

Conclusion: These data reveal that similar monoclonal antibodies targeting MPR have been isolated from multiple individuals with different HIV subtypes. These mAbs, VRC42.01 (subtype CRF01_AE), PGZL.1 (B) and now MHRP.01 (B) have identical gene usage, targeted epitope, angle of binding, and neutralization profiles, suggesting that individuals from different demographic areas could elicit B-cell clones targeting this epitope through vaccination efforts.

Structural comparisons of MHRP.01 with 4E10 and VRC42.
ART-DIX: A NOVEL STRATEGY TO MONITOR BROADLY NEUTRALIZING AND s
AFTER pH-DEPENDENT DISSOCIATION OF PLASMA PROTEINS (ART-DIX:
WE DEVELOPED A NOVEL ARV REMOVAL STRATEGY TO SEPARATE ARVS BY SIZE EXCLUSION
TUNED THE PURIFICATION OF PLASMA ABS BY PROTEIN A/G TO ASCERTAIN FULL AB RECOVERY.
CONFORMATIONS IN THE VSV CONTEXT. NEXT, TO REMOVE ARVS FROM PLASMA, WE FINELY-
TO ARVS PROVED NOT SATISFACTORY AS VSV-BASED ENV PSEUDOTYPES DISPLAYED
SEVERELY. EXPLOITING THE INHERENT RESISTANCE OF VESICULAR STOMATITIS VIRUS (VSV)
WITH MULTIPLE ART-RESISTANCE MUTATIONS (HIV-LUC-RES). HOWEVER, FULL RESISTANCE
SCREENING. TO GENERATE ART-RESISTANT VIRUSES, WE DEVELOPED NLUC VARIANTS
COMPARED ALL FOUR METHODS FOR EFFICACY AND SUITABILITY FOR HIGH-THROUGHPUT
REMOVING ARVS. WE USED TWO DIFFERENT APPROACHES FOR EACH STRATEGY AND
INVESTIGATED TWO BASIC STRATEGIES, NAMELY USING ART-RESISTANT VIRUSES OR
RESULTS:

MATERIALS AND METHODS:

THE ART-COMPATIBLE NEUTRALIZATION ASSAYS WERE CONDUCTED
ON TZM-BI CELLS AND COMPA The standard NLuc-based pseudovirus neutralization assay. We included 13 multi-clade viruses and MuLV as control. INHIBITION IN PRESENCE OF COMMON ARVS, BNABS, AND PLASMA FROM ART-TREATED patients (N=23) was assessed.
RESULTS: TO ELIMINATE THE IMPACT OF ART ON NEUTRALIZATION ASSAYS, WE
INVESTIGATED TWO BASIC STRATEGIES, NAMELY USING ART-RESISTANT VIRUSES OR
REMOVING ARVS. WE USED TWO DIFFERENT APPROACHES FOR EACH STRATEGY AND
COMPARED ALL FOUR METHODS FOR EFFICACY AND SUITABILITY FOR HIGH-THROUGHPUT SCREENING. TO GENERATE ART-RESISTANT VIRUSES, WE DEVELOPED NLuc VARIANTS WITH MULTIPLE ART-RESISTANCE MUTATIONS (HIV-LUC-RES). HOWEVER, FULL RESISTANCE WAS NOT ACHIEVED AS COMBINED RESISTANCE MUTATIONS AFFECT INFECTIVITY TOO SEVERELY. EXPLOITING THE INHERENT RESISTANCE OF VESICULAR STOMATITIS VIRUS (VSV) TO ARVS PROVED NOT SATISFACTORY AS VSV-BASED ENV PSEUDOTYPES DISPLAYED INCREASED neutralization sensitivity to certain Abs, suggesting more open trimer conformations in the VSV context. Next, to remove ARVs from plasma, we fine-tuned the purification of plasma Abs by protein A/G to ascertain full Ab recovery. HOWEVER, WHILE THE APPROACH WAS SUCCESSFUL, IT IS NOT SCALABLE AND COSTLY. FINALLY, WE DEVELOPED A NOVEL ARV REMOVAL STRATEGY TO SEPARATE ARVS BY SIZE EXCLUSION AFTER pH-DEPENDENT DISSOCIATION OF PLASMA PROTEINS (ART-DIX: DISSOCIATION AND sSIZE-EXCLUSION). ART-DIX PROVED TO BE HIGHLY EFFECTIVE IN REMOVING ARVS, WITH ONLY THE MOST POTENT DRUGS SHOWING SOME RESIDUAL ACTIVITY THAT CAN BE OVERCOME WHEN USING ART-DIX IN COMBINATION WITH HIV-LUC-RES VIRUSES.
CONCLUSION: THE METHOD PORTFOLIO PRESENTED HERE OFFERS VERSATILE OPTIONS TO REDUCE CONFOUNDBING EFFECTS OF ARVS IN neutralization assays. Depending on the ART scheme, research question, and required throughput, alternative test formats may prove beneficial.
**308 ASSESSING IMMUNOGENICITY BARRIERS OF THE HIV-1 ENVELOPE TRIMER**

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**Background:** Understanding the counterbalance of epitope shielding and accessibility on HIV-1 envelope (Env) trimers is essential to guide immunogen selection for broadly neutralizing antibody (bNAb) based vaccines. To investigate the antigenic space of highly stabilized Env trimers, we here created a novel strategy, DARPin Antigenicity Analysis (DANA), based on synthetic, high diversity Designed Ankyrin Repeat Protein (DARPins) libraries. We show that DANA, a purely in-vitro screening tool, has the means to inform on the antigenic properties of Env immunogens by recapitulating the difficulty of the human immune system to generate antibodies recognizing pseudofur clover Env in vivo.

**Methods:** High diversity DARPin libraries consist of ~190Da-sized synthetic proteins with a consensus-designed framework and randomized residues in the binding surface. We performed 12 independent DANA screens using different SOSIP-type Env trimers as panning targets to select Env-specific DARpins from DARpin libraries by ribosome display. 190 DARpins from each DANA screen were sequenced and analyzed for trimer and V3 loop reactivity by ELISA and neutralization breadth using a 5-virus panel in the standard TZM-bl based pseudovirus neutralization assay.

**Results:** Comparison of Env trimers in DANA screens revealed that stronger trimer stabilization, including the V3, lead to the selection of highly mutated DARpins with length variations and framework mutations. This is highly reminiscent of the extensive affinity maturation and framework mutations reported for bnAbs, indicating that DARpins and antibodies, despite their genuinely different binding architecture, have the same underlying biophysics of interaction and thus similar limitations in recognizing the shielded trimers. Notably, by exploring different selection regimens, we showed that in DANA reduction of V3 dominance benefits the selection of trimer-reactive clones, reaffirming current concepts of vaccine design. As we show, DANA screens have, next to investigating single immunogens, the capacity to mimic heterotypic prime-boost immunization regimens and to select immunogen combinations that favor the selection of trimer reactive binders.

**Conclusion:** Our results demonstrate the utility of DANA screens as a versatile tool for basic antigenicity screening of immunogen candidates, which may substitute for some of the early-stage animal testing required in vaccine development.

**309 ISOLATION OF PAN-SARBEVIRUS mAbs THAT NEUTRALIZE SARS-CoV-1 AND SARS-CoV-2 VARIANTS**

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**Background:** The continued emergence of severe acute respiratory syndrome coronaviruses (SARS-CoVs) and recent exploitation of the SARS-CoV-2 pandemic highlights the need for broad and potent antibody recognition and understanding the contexts in which they may develop. Antibodies with cross reactivity across SARS lineages may be of particular value in preparing for future pandemic highlights the need for broad and potent antibody recognition and understanding the contexts in which they may develop.

**Methods:** We isolated monoclonal antibodies (mAbs) from an individual infected with SARS-CoV-2 with a severe illness, followed by convalescence. Immunofluorescence staining and plaque reduction neutralization assays were used to identify neutralizing antibodies. We performed an ELISA for binding and neutralization of Spike trimers and pseudoviruses. We also performed deep mutational scanning (DMS) to identify mutations that affect antibody binding. Lastly, binding breadth was further evaluated using a yeast display library of RBDs from diverse SARS-CoV-1-related CoVs and African and European sarbecoviruses isolates as well as SARS-CoV-2 VOCs.

**Results:** We describe mAbs from a SARS-CoV-2-infected individual that bound and neutralized both SARS-CoV-2 and SARS-CoV-1, including one that showed breadth across recent VOCs. Given their breadth, these SARS-CoV-2 cross-reactive mAbs may be robust to viral escape and thus could contribute to therapeutic efforts. In addition, these mAbs displayed broad cross-reactive activity across sarbecoviruses and may be beneficial against future spillover events.

**310 BROAD ANTIBODIES TO FUNCTIONALLY CONSTRAINED REGIONS OF SARS-CoV-2 SPIKE**

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**Background:** While remarkable and rapid progress was made in fighting the SARS-CoV-2 pandemic with vaccines and therapeutic antibodies, these approaches were quickly compromised by viral evolution. Therapeutic monoclonal antibodies (mAbs) that were once authorized for clinical use, which all target the receptor binding domain (RBD), are no longer effective against current variants of concern (VOCs) due to mutations in this region of Spike. Thus, to achieve durable protection against SARS-CoV-2, novel mAbs need to show breadth and potency across VOCs and target epitopes that are more constrained.

**Methods:** mAbs from an individual who had a breakthrough Delta Spike infection after vaccination were isolated from Spike-specific memory B cells. mAbs were assessed for binding affinity and neutralization potency using Spike-pseudotyped lentivirus (PSV) and live SARS-CoV-2 virus neutralization assays. Epitopes were mapped using deep mutational sequencing (DMS) and structural-based methods.

**Results:** Three novel mAbs (C68.3, C68.13, C68.59) demonstrated binding breadth to Spikes from various VOCs including Omicron VOCs despite that C68 had not yet been exposed to Omicron. These mAbs potentially neutralized the Wuhan-Hu-1 vaccine and Delta strains (IC50 = 9-61ng/mL), and early Omicron strains BA.1, BA.2, BA.5 (IC50 = 12-149 ng/mL). C68.3 and C68.59 retained potency against recent VOCs BA.1.1 and XBB (IC50 = 121-122 ng/mL and 56-82 ng/mL, respectively) in the PSV assay. Similar neutralization activity was observed in the live virus assay. The potency of these mAbs was greater against Omicron VOCs than all but one of the mAbs previously authorized for treatment and they showed greater breadth. The mAbs target distinct epitopes on the Spike glycoprotein, two in the RBD (C68.3, C68.13) and one in an invariant region downstream of RBD in subdomain 1 (SD1) (C68.59). Structural analysis of C68.59 Fab binding to Spike trimer revealed significant allosteric changes to regions of Spike outside of the epitope in the 2u unit. Finally, DMS escape pathways showed these mAbs target regions highly conserved across VOCs that are also functionally constrained, suggesting escape could incur a fitness cost.

**Conclusion:** Overall, these mAbs are novel in their breadth across VOCs and include a potent mAb targeting a rare epitope outside of the RBD in SD1. These mAbs focus on diverse, functionally constrained regions in Spike making them candidates for development as combination therapeutics with good durability against future VOCs.

**311 IL-15 DEPENDENT NK CELLS AND DENDRITIC CELLS CROSS-TALK IN HIV-1 ELITE CONTROLLERS**

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**Background:** HIV-1-specific cell responses are commonly regarded as the backbone of antiviral immunity in HIV-1 elite controllers (ECs), while the specific
contribution of innate immune cells is less clear. Natural Killer (NK) cells are of particular interest, as they can mount direct cytotoxic effects against HIV-1-infected cells that do not depend on MHC class I restriction and are less prone to viral mutational escape. Here, we investigated epigenetic, transcriptomic and metabolic characteristics of NK cells from ECs.

**Methods:** Epigenomic, transcriptomic and metabolic profiles of sorted CD56<sup>dim</sup> CD16+ NK cells from HIV-1 ECs (n=20) were compared to antiretroviral-treated HIV-1-infected individuals (HAARTs; n=21) and HIV-1 negative healthy donors (HIVNs; n=18). Cut&RUN-seq was used to assess DNA segments bound to activating and inhibitory histone modifications, while transcriptional signatures were analyzed by RNA-Seq. Seahorse assays were used to evaluate metabolic activities of NK cells.

**Results:** Compared to reference cohorts, epigenomic profiles of ex vivo sorted CD56<sup>dim</sup> CD16+ NK cells from ECs showed statistically significant differential enrichments, with the activating histone H3K27ac and H3K4me3 marks in S46 and 391 gene loci, respectively. However, no differences were seen for the inhibitory H3K27me3 mark. Notably, within ECs, the IL-2Rβ gene was enriched with activating histone modifications, and was more strongly expressed on the transcriptional and the protein levels in CD56<sup>dim</sup> CD16+ NK cells. Correspondingly, IL-15, the physiological ligand for the IL-2Rβ chain, was more strongly expressed by myeloid dendritic cells (mDCs) from ECs compared to alternative study cohorts, and transcriptional signature pathways showed evidence for an improved cross-talk between mDCs and NK cells from ECs. Upon IL-15 stimulation to NK cells, an upregulation of the anti-apoptotic molecule BCL2 was seen in activated CD69+ CD56<sup>dim</sup> CD16+ NK cells from ECs, compared to HAARTs (p=0.011) and HIVNs (p=0.001). Furthermore, IL-15-stimulated NK cells from ECs displayed an elevated level of glycolysis, compared to the other cohorts.

**Conclusion:** CD56<sup>dim</sup> CD16+ NK cells from ECs are epigenetically poised to mount improved responses to IL-15 stimulation, allowing for improved survival and optimized metabolic activities. We propose that a distinct immune cross-talk between NK cells and mDCs may reflect features of trained innate immunity in ECs.

**312 DISTINCT NK CELL RESPONSES DEFINE DURABLE CONTROL IN ELITE CONTROLLERS**

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**Background:** Elite Controllers (EC) are PLWH that present drug-free control of the infection, representing a model for a functional cure. Hence, understanding their mechanisms of immune-mediated control is of considerable interest. We sought to characterize the NK cell repertoire in EC, including their memory-like properties and functional potential.

**Methods:** PBMC samples from n=33 EC (n=20 with durable control (DC), and n=13 with immunological aborted control (AC), defined by a progressive CD4+ T cell depletion (p=0.05) during the patient’s follow-up), n=25 healthy donors (HD), n=8 ART-suppressed (ART), and n=7 viremic (VIR) participants were included in the study. Phenotypic studies were performed by flow cytometry and included the markers CD57, CD65, Nkp30, NKG2A, NKG2D, CD16, CXCR3, KIR2DL2/3, and KLRG1. Natural cytotoxicity and cell activation were evaluated by IFN-γ, CD107a, CD69 and HLA-DR expression by flow cytometry after co-culturing isolated NK cells with the latently-infected ACH-2 cell line and HIV+ plasma. P24 expression was measured by flow cytometry.

**Results:** NK cells from DC presented lower NKG2A expression (p=0.004) and basal cell activation, based on HLA-DR (p=0.033) and CD69 (p=0.042) expression, compared to AC. In addition, NKG2A+ NK cells, associated to memory-like NK cells, were expanded in DC compared to HD and AC (p<0.001 and p<0.040), and were found to produce higher basal IFN-γ levels than AC (p=0.024). Although NK cells from DC showed a marked cytotoxic response to K562 in the presence (p<0.001) or absence of IL-15 (p<0.001) compared to unstimulated conditions, the response was significantly lower compared to HD (pK562=0.001, pIL-15=0.049). However, NK cells from DC showed a strong ADCC response, similar to HD. Importantly, DC presented a remarkable ADCC response compared to AC (p=0.003) and VIR (p=0.013) participants (Figure 1A), which correlated positively with KLRG1 (p=0.008, r=0.57) and NKG2D expression (p<0.001, r<0.52) and inversely with the cytotoxic response presented upon K562 (p<0.001, r<0.80) and IL-15 (p<0.003, r=0.74) stimulation (Figure 1B).

**Conclusion:** Our findings suggest that durable immune-mediated control of HIV infection in EC is defined by NK cell phenotypic and functional attributes, including lower cell activation, an increased memory-NK cell compartment and higher ADCC responses.

Figure 1. ADCC responses are associated to phenotypical and functional changes in the NK cell repertoire. (A) Summary graph of the ADCC activity mediated by NK cells from study groups in the presence of plasma from a viremic HIV+ participant. Statistical comparisons were performed using Kruskal-Wallis One-way ANOVA followed by Dunn’s multiple comparisons test. **p<0.001, ***p<0.001, ****p<0.0001. (B-C) Heatmaps summarizing the correlations between ADCC responses and (B) NK cell receptor expression (left to right: CD158b, CXCR3, KLRG1, NKG2A, NKG2D, Nkp30, NKG2C and CD57) in CD56total NK cells or (C) functional markers (IFN-γ, CD107a, HLA-DR and CD69) in (left to right): unstimulated, K562-stimulated and IL-15-K562-stimulated CD56total NK cells by study group. Statistical analysis was performed using two-tailed spearman rank correlation. **p<0.05, ***p<0.001, ****p<0.0001.

**313 MONOCYTES OF HIV-1 ELITE CONTROLLERS AND RELATIVES SHOW ENHANCED TRAINED IMMUNITY**

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**Background:** Less than 1% of people living with HIV (PLHIV) spontaneously control HIV-1. Characterization of immune drivers eliciting this spontaneous control remain important. Monocytes can undergo long-term functional reprogramming after exposure to certain stimuli such as β-glucan, a process called trained immunity (TI), which confers heterologous protection against multiple pathogens. We hypothesized that spontaneous controllers show enhanced innate immune responsiveness and TI compared with noncontrolling PLHIV. Immune stimulations in the 1st degree relatives of both groups were used as surrogates of the immune reactivity without HIV.

**Methods:** In a double case control design, HIV-1 elite controllers (EC; N=31) and non-controlling PLHIV on suppressive antiretroviral therapy (ART) (non-EC; N=30) were recruited, as well as relatives from both groups (N=23 and N=22, respectively). Groups were similar in age, sex, BMI and vaccination history. EC were subdivided into non-viremic (N = 17) and viremic (N=14), based on viral load cutoffs < 75 and < 10,000 copies/mL, respectively. Adherent monocytes were exposed ex vivo to 1 ug/ml β-glucan (trained) or RPMI (control) for 24 hours at 37°C. After 24 hours, the cells were washed and left to rest for 5 days,
Followed by stimulation with 10 ng/mL LPS on day 6, TNF, IL-6 and IL-1RA were measured in supernatant at day 7 by ELISA.

**Results:** EC relatives had stronger TNF, IL-6 and IL-1RA production capacity of control monocytes after LPS stimulation than non-EC relatives (P = 0.0013, P = 0.013 and P = 0.013, respectively, Fig 1A). Induction of trained immunity was also stronger in EC relatives after b-glucan exposure and LPS stimulation (P = 0.0021, P = 0.078 and P = 0.0056 for TNF, IL-6 and IL-1RA, respectively, Fig 1B). Control monocytes responses of EC and non-EC PLHIV were similar after LPS stimulation (P = 0.54, P = 0.63 and P = 0.063 for TNF, IL-6 and IL-1RA, respectively). However, trained monocytes of EC showed greater induction of TNF, IL-6 and IL-1RA production (P = 0.047, P = 0.0054 and P = 0.043, respectively, Fig 1C). Both trained and control monocytes of non-viremic EC produced more TNF than viremic EC (P = 0.012 and P = 0.012, respectively, Fig 1D).

**Conclusion:** Elite controllers and their first-degree relatives show both increased innate immune responses and trained innate immunity compared to non-controlling PLHIV on ART and their first-degree relatives. Further studies on the role of trained innate immunity to control HIV-1 are warranted.

**314 TRAINED IMMUNITY FEATURES IN NK CELLS OF HIV-1 ELITE CONTROLLERS**

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**Background:** Recent work has shown that a subset of ECs have distinct viral reservoirs where CD57highNKG2ChighKIR2low NK cells were only present in CMV-seropositive individuals. A high capacity for target cell-induced IFNγ production has been attributed to these NK cells. Increased NKG2C expression together with enrichment of H3K4me3 at the promoter of KLRC2 gene, the coding gene for NKG2C, has been attributed to these NK cells. Increased NKG2C expression together with enrichment of H3K4me3 at the promoter of KLRC2 gene, the coding gene for NKG2C, was observed in 3 EC in comparison to 3 non-EC (Fig 1C). An increased H3K4me3 enrichment at the promoter of KLRC2 gene, the coding gene for NKG2C, was observed in 3 EC in comparison to 3 non-EC (Fig 1C).

**Conclusion:** Unsupervised analysis revealed the presence of two distinct memory HNK subpopulations in PLHIV. CD57highNKG2ChighKIR2high NK cells were specific to PLHIV and had greater NKG2C and ILT2 expression in EC. CD57highNKG2ChighKIR2low NK cells were only present in CMV-seropositive individuals. A high capacity for target cell-induced IFNγ production has been attributed to these NK cells. Increased NKG2C expression together with enrichment of H3K4me3 at the promoter of KLRC2 gene suggests improved antiviral control in ECs through induction of trained immunity.

**315 ELITE CONTROLLERS WITH DISTINCT VIRAL RESERVOIR HAVE ROBUST CTL TO NETWORKED EPITOPES**

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**Background:** A major objective for HIV cure research is treatment-free suppression of infection, which has been achieved by individuals who spontaneously control HIV to undetectable levels (elite controllers, ECs). Prior analyses of ECs demonstrated that functional cytotoxic T lymphocyte (CTL) responses towards epitopes derived from mutationally constrained (‘networked’) regions of the HIV proteome were a key distinguishing feature. Recent work has shown that a subset of ECs have distinct viral reservoirs where integrated near-full-length HIV genomes are primarily within transcriptionally repressive chromosomal locations, representing an even more promising model of HIV cure, but for which immune correlates remain to be defined. Thus, we characterized HIV-specific CTL responses in ECs with distinct viral reservoirs (DVRs).

**Methods:** The specificity and functionality of HLA-restricted HIV-specific CTL responses in 10 ECs with DVRs (Jiang et al, Nature 585:261) and 30 HIV+ individuals on long-term ART by IFN-g ELISpot, proliferation assay, T cell phenotyping and CTL elimination assay. Matched integration and proviral sequencing was performed on the entire cohort and was used to evaluate the sequence diversity of targeted epitopes.

**Results:** Evaluation of CTL immunodominance hierarchies by IFN-gamma ELISpot and tetramer staining revealed preferential targeting of networked epitopes by ECs with DVRs in comparison to individuals on ART (p < 0.05). Further assessment of the functionality of CTL responses targeting networked epitopes revealed highly significant differences in proliferative capacity (p < 0.0001) and primary CD4+ T cell elimination (p < 0.0001), with some proliferative networked CTL responses being among the highest observed to date. Evaluation of viral sequence diversity in near full-length provirus revealed an absence of immune escape mutations within networked epitopes despite the presence of highly functional CTL responses.
Conclusion: Our findings demonstrate that ECs with distinct viral reservoirs have highly functional CTL responses against networked epitopes in comparison to individuals on long-term ART. Moreover, these networked epitopes resist mutagenesis despite robust CTL pressure. These studies therefore indicate that induction of proliferative and cytotoxic T cell responses targeting mutually constrained regions of HIV may be a viable strategy to restrict proviruses to transcriptionally repressed genomic regions and thereby durably suppress virus-producing cells.

DNA LAUNCHED BROADLY NEUTRALIZING KILLERS: A DOUBLE-EDGED THERAPEUTIC AGAINST HIV-1

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Background: Robust induction of CD8+ T cells play important role in control of HIV-1 viremia. Here, we describe design and implementation of approaches to recruit cytotoxic T-cell responses for achieving HIV-1 clearance. We have exploited a combination of two critical immune components, first is the HIV virus neutralization property of the Broadly Neutralizing Antibodies (bNAbs) and second — the killer potential of cytotoxic T cells as DNA launched therapeutic intervention against HIV-1.

Methods: We designed DNA launched broadly neutralizing killers (bNKs) which are single chain molecules, these engage CD3+ T cells from one arm and HIV-1 infected target cells using the other arm. Multiple bNKs for targeting various sites of vulnerabilities present on HIV-1 Env protein like CD4bs, V1/V2 loop, etc. have been developed. We evaluated the expression profiles of these DNA launched bNKs and characterized for functionality in terms of binding, neutralization and killing by recruitment of effector cells first in vitro and then in vivo. ELISA and flow cytometry was employed for binding studies, followed by pseudo-neutralization assays to test neutralizing potential of these therapeutics against global virus panel. A novel cell based Xcelligence assay was designed to use impedance as a readout for killing activity of bNKs in the presence of serum expressing the bNKs exhibited cytolysis and killing of HIV-1 infected cells in novel cell based Xcelligence assay. Our data highlights the design and characterization of novel DNA launched bNKs which are potent double-edged therapeutics capable of broadly neutralizing difficult to neutralize Tier2/3 viruses with high potency and specificity. These were found to be extremely potent killers against HIV-1 infected target cells with picogram IC50 for killing. Further, IM administration in mice using electroporation was performed and the serum expressing the bNKs exhibited cytolyis and killing of HIV-1 infected cells in novel cell based Xcelligence assay.

Conclusion: Our data highlights the design and characterization of novel DNA launched bNKs which are potent double-edged therapeutics capable of broadly neutralizing difficult to neutralize Tier2/3 viruses and engaging effector T cells to their targets and mediate killing of HIV-1 infected cells with high potency and specificity. Further designs are being currently explored to use these molecules as combination therapies to engage various other effector cells of the immune system and target arresting HIV-1 virus escape using our robust HIV therapy.

CD8 T cells for their phenotypic and functional profiles using multicolor flow cytometry. We performed immunohistochemistry IHC to stain for LAIR-1 in LN tissues and used RNA sequencing to study the transcriptomic profile of LAIR-1+ CD8 T cells.

Results: Here, we report on the expression of LAIR-1 on virus-specific CD8+ T cells during chronic SIV infection and the effect of LAIR-1 blockade on the proliferation and cytokine function of these cells. LAIR-1 expression was found to be low on naive CD8+ T cells and increased on total and SIV-specific effector memory CD8+ T cells during chronic infection. In addition, the level of LAIR-1 expression in the lymph node during chronic infection was higher compared to naive animals. Transcriptionally, these LAIR-1+ CD8 T cells exhibited a unique transcriptome characterized by with heightened type I IFN-signaling coupled with reduced TCR signaling, cell cycling and cell metabolism pathways. Importantly the expression of LAIR-1 on SIV-specific effector memory CD8+ T cells correlated directly with the SIV viral RNA levels in plasma. Indeed, in vitro blockade of LAIR-1 using LAIR-1-Fc/anti-LAIR-1 antibody resulted in enhanced proliferation of SIV-specific CD8 T cells with cytotoxic function.

Conclusion: These results serve as a foundation for future in vivo trials of the use of LAIR-1 blockade to potentially enhance and/or restore antiviral SIV-specific CD8 T cells, especially in secondary lymphoid tissues which may be important for the HIV cure strategy.

LAIR-1 mediated inhibition of CD8 T cells during chronic SIV/HIV infection

TIGIT/CD155 BLOCKADE BOOSTS T-CELL IMMUNITY AND HIV-SPECIFIC DEGRANULATION IN PLWH

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Background: Exploring immune interventions, such as immune checkpoint blockade, is critical to attenuating the HIV cure. To date, these strategies focus on a narrow set of targets using conventional monoclonal blocking antibodies. Although several studies have indicated the potential of targeting the TIGIT inhibitory pathway in people living with HIV (PLWH), no focus has been paid to its ligand CD155. Here, we propose to explore CD155 as an immunoregulatory target in PLWH.

Methods: We engineered two soluble Inhibitory Receptors (sIRs) proteins (monomeric sIR1 and Fc-dimeric sIR2) with a sIR-control. We determined sIRs affinity to CD155 by SPR and evaluated CD155 blockade in a coculture using a Jurkat-TIGIT+ and BVS417/OKT3-CD155+. Moreover, we assessed CD155 blockade by sIRs in PBMCs from PLWH on ART (n=17). Briefly, PBMCs were stimulated with sIRs in the absence or presence of an HIV-1 Gag peptide pool. After 16h, we analyzed antigen-independent and HIV-specific responses by flow-cytometry combining CD3, CD4, CD8, CD45RA, CCR7, CD16, CD56, CD155, TIGIT, IFNg, TNF, CD107a, IL-2, and IL-10 markers.

Results: We determined sIR-specific binding to CD155 and found an increased activation in sIR2 (8.6nM) compared to sIR1. In coculture, only the blockade of CD155 with sIR2 increased the activation of NFkB (p=0.034) and the coactivation of NFkB/NFAT (p=0.006) in Jurkat-TIGIT+ cells. TIGIT blockade by mAbs only produced NFkB/NFAT coactivation (p=0.0045). Using PBMCs from PLWH, we found in an antigen-independent manner, increased production of IFNg (p=0.002), TNF (p=0.003), IL-2 (p=0.02) and IL-10 (p=0.0009) in CD8+ T-cells and increased production of IL-2 (p=0.055) and IL-10 (p<0.0001) in CD4+ T-cells by sIR2. In addition, sIR2 increased the frequency of TIGIT+CD8+ (p<0.0001) and TIGIT+CD4+ (p=0.002) T-cells with augmented degranulation capacity.
However, the increase of TIGIT+ T-cells did not correlate with loss of IFNγ, TNF, IL-2, IL-10 or CD107a production. In HIV-1 stimuli, sIR2 favored a specific increase of CD107a in TIGIT+CD8+ (p=0.003) and TIGIT+CD4+ (p=0.003) T-cells in the absence of IFNγ, TNF, IL-2, and IL-10 production. No effect was found for sIR1.

Conclusion: We generated sIRs immunomodulatory proteins capable of binding CD155. Our data demonstrate blockade of CD155 and enhancement of T-cell cytokine production by sIR2. Moreover, sIR2 increased HIV-specific degranulation in TIGIT+CD4+ and TIGIT+CD8+ T-cells in PLWH on ART. Thus, we propose CD155 as a potential target for novel immunotherapeutics in PLWH.

EVALUATION OF HIV-SPECIFIC T CELL RESPONSE IN BEAT2 CLINICAL TRIAL

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Background: Passive administration of HIV-1 broadly neutralizing antibodies (bNAbs) can achieve durable viral suppression when replacing ART. Previous studies have suggested that ART substitution with bNAb administration can have a vaccinal effect on HIV-specific T cells, increasing response frequencies and improving functional properties during bNAb-mediated suppression. Here, we evaluated whether such a vaccinal effect occurred in the BEAT2 clinical trial, which tested a 26-week combination of bNAbs (3BNC117 and 10-1074) and peg-ifn-α2b (IMM-Tx) off-ART, followed by an off-IMM-Tx follow-up non-intervention ATI (non-int. ATI).

Methods: Cryopreserved peripheral mononuclear cells were obtained from the BEAT2 study (NCT03358875) in which baseline bNAb-sensitive PLWH received 29 weekly doses of peg-ifn-α2b (1.5 µg/kg) and 26 wks off ART, and seven IV infusions of the bNAbs 3BNC117 and 10-1074 (30 mg/kg during the 26 wks off ART). Ten participants received combined IMM-Tx, and one received only bNAbs infusions. Activation-induced marker (AIM) assay and flow cytometry were used to quantify HIV-specific immune responses in all 11 donors at several time points, including baseline on ART (on), after 4x weekly peg-ifn-α2b on ART, after 26 weeks of IMM-Tx (7 bNAbs infusions plus peg-ifn-α2b), and during the final non-int. ATI. HIV-1 specific CD4+ or CD8+ T cells were identified as CD69+PD1+ or CD69+CD137+ or PD1+CD137+ after stimulation against HIV-1 Consensus B gag peptide pool.

Results: We found no increase in gag-specific CD4+ and CD8+ T cell responses during IMM-Tx (with or without peg-ifn-α2b) compared to baseline in most donors; increased CD8+ responses after peg-ifn-α2b were observed in a single participant. However, we did find an increase in the proportion of gag-specific CD4+ and CD8+ T cells in 4 out of 11 individuals during the post-IMM-Tx non-int. ATI compared to earlier time points. These individuals showed sustained control of viremia (Viral load <20 c/ml for 1), suggesting an association between emerging T cell response and control of the viremia.

Conclusion: We found no detectable change in HIV-specific T cell responses after 26 weeks of IMM-Tx with 3BNC117+10-1074 bNAbs plus peg-ifn-α2b immunotherapy; however, an increase in gag-specific T cell responses was associated with viral control following the end of IMM-Tx in a subset of persons, suggesting a potential link between IMM-Tx and cell-mediated responses in viral suppression.

ONE-YEAR TREATMENT WITH PONATINIB INDUCES SUSTAINED CYTOTOXIC ACTIVITY AGAINST HIV

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Background: Treatment with tyrosine kinase inhibitors (TKIs) protects CML who were treated with ponatinib for one year as consolidation therapy. We evaluated the persistence of HIV-infected cells. PLWH on ART and dasatinib present reduced CD4 cells from HIV infection, inducing functional cytotoxic populations that can eliminate HIV-infected cells. We evaluated the persistence of HIV-1 reservoir that is resistant to reactivation. We evaluated the persistence of HIV-infected cells. PLWH on ART and dasatinib present reduced CD4 cells from HIV infection, inducing functional cytotoxic populations that can eliminate HIV-infected cells. PLWH on ART and dasatinib present reduced CD4 cells from HIV infection, inducing functional cytotoxic populations that can eliminate HIV-infected cells.

Methods: Nine participants of Phase II clinical trial NCT04043676 were recruited. They achieved deep molecular response against CML after 14 (IQR 5.5-15.5) years of treatment with imatinib before interruption and then received one-year consolidation treatment with ponatinib 15 mg/day. Blood samples were taken before starting ponatinib (t=12 months), after one year of treatment (t=0), and 3, 6 and 12 months after interruption. PBMCs nrtivital activity was evaluated by measuring caspase-3 activity of NL4.3 retrovirus-infected TZM-bl cells. Cytotoxic cell populations were characterized by flow cytometry.

Results: 1) 5 participants (55.5%) did not relapse from CML 12 months after ponatinib interruption (Non-relapsed), while 4 participants (44.4%) relapsed after 5.5 months (IQR 4.25-6.75) of ponatinib interruption (Relapsed). 2) PBMCs from Non-relapsed showed 2-fold increased viral cytotoxicity (p=0.0317) after 1-year of ponatinib, in comparison with Relapsed (Figure). Cytotoxic activity was similar in both groups 3-months after interruption and remained increased 1.7- and 1.3-fold for 5.5 and 11 months, respectively, in Non-relapsed after ponatinib withdrawal. 3) Degranulation activity (CD107a+) of CD8 was increased 4.08-fold (p=0.0317) in Non-relapsed and it was maintained 3-months after interruption. 4) CD8+ TCRgd+ cells increased in both groups (p=0.0330) since treatment withdrawal but their degranulation capacity was only significantly increased 3.3-fold (p=0.0078) after ponatinib withdrawal in Non-relapsed. 5) NK cell count (CD3+CD56+) sustainability increased in Non-relapsed (p=0.0039); degranulation capacity of NK cells (CD3+CD56+) was increased in Non-relapsed (p=0.0078).

Conclusion: Potent, sustained cytotoxic antiviral response against HIV-infected cells was induced after one-year treatment with ponatinib in non-relapsed individuals. Temporary intensification treatment with TKIs such as ponatinib could stimulate the antiviral response in PLWH during cure strategies.

DECREASED HIV-SPECIFIC POLYFUNCTIONAL RESPONSES FOLLOWING DIPYRIDAMOLE TREATMENT

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Background: Decreases in extracellular adenosine (ADO) production are associated with increased inflammation and immune activation in ART-treated people living with HIV (PWHA). We previously showed that 12 weeks (w) of treatment with dipyriramole (DP), which increases extracellular ADO levels, decreased CD8+ T cell activation in PWHA. Here, we investigated if the decrease in activation also affected HIV-specific T cell responses.

Methods: PWHA with viral suppression on ART were randomized to 12w of DP vs placebo (PL). Peripheral blood mononuclear cells were obtained pre- and post- DP treatment. Using flow cytometry, we evaluated changes in Gag- and Env-specific polyfunctional T cell responses (CD107a, TNF, IFNy, IL2, Granzyme B). We measured purine levels, soluble markers of inflammation, T cell immune activation (HLA-DR/CD38+) and cell cycling (Ki-67), and residual viremia by single copy assay to determine whether immune responses were associated with these parameters (using Spearman).

Results: Twenty of 40 participants enrolled (9 DP, 11 PL) had available specimens for this subs study (90% male; median age 57; median CD4+ = 673 cells/mL; median 17 years on ART). There was a significant decrease in Gag-specific (% change from baseline (BL) to w12 -63.18% DP vs -28.14 PL; p<0.001); Mann Whitney and Env-specific (-57.5% DP vs -16.0% PL; p=0.025) CD4+ polyfunctional response (2 or more immune mediators expressed). No differences in HIV-specific CD8+ T cell responses were observed. Higher levels of intron, a surrogate of ADO, at w12 was associated with decreased CD4+ Gag-specific polyfunctional responses at w12 compared to BL (r=-0.56; p=0.04).

Although there were no overall differences in BL to w12 changes in cell cycling, change in the frequency of CD4+ and CD8+ cell cycling positively correlated
Results: All regimens were well tolerated with transient small increases in inflammatory markers and body temperature. The vaccine elicited strong IFNγ ELISPOT responses 4 weeks post-ChAd prime (8.724 ± 1.845 SFU/10⁴ PBMCs, mean ± SEM), which were further augmented by boosts with samRNA (mean 1.8, 3.7, and 11.5-fold increase post each boost, respectively) and ChAd (1.7-fold increase). The responses peaked at 32, 23,44 ± 4,433 SFU/10⁴ PBMCs (mean ± SEM) and were durable through at least 16 weeks post last immunization (range 3,398 – 15,360 SFU/10⁴ PBMCs). Combination with either aP0-1, aCTLA-4 or FLTR3a further augmented mean T cell response magnitude by 2.8, 2.4 and 5.7-fold, respectively. When assessed using antigen-derived overlapping peptides, the breadth of responses at week 13 was greater in combination with aCTLA-4 or FLTR3a than with vaccine alone (p < 0.05). Vaccine alone elicited predominantly CD8+ T-cell responses (1.9% IFNγ+/CD8+ and 0.6% IFNγ+/CD4+, week 13), while FLTR3a also robustly enhanced CD4+ T-cell responses (2.0% IFNγ+/CD8+ and 3.5% IFNγ+/CD4+, week 13). T cell responses were polyfunctional (predominantly IFNγ+ + TNFα+) in all groups.

Conclusion: ChAd/samRNA heterologous SIV vaccine was well tolerated and induced robust and broad antigen-specific T cell responses. The immune responses were augmented by aP0-1, aCTLA-4 or FLTR3a warranting their further exploration as part of a combination therapeutic approach for HIV cure. Figure 1. Strong and durable SIV-specific T cell response in rhesus macaques following vaccination with ChAd/samRNA is enhanced by co-administration of aP0-1, aCTLA-4 or FLTR3a. IFNγamma ELISPot, sum of Env, Pol and Gag overlapping peptide pools, background subtracted. Mean ± SEM. Green triangles represent ChAd immunizations and red squares represent samRNA immunizations.

324 CD40.HIVRI.Env VACCINE INDUCES STRONG AND DURABLE IMMUNE RESPONSES: ANRS VRI06 TRIAL

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Background: RV144 study identified anti-HIV Env V1/V2 binding IgG and Env-specific CD4+ T cells as immune correlates of protection. We tested here a new way of antigen (Ag) delivery to improve vaccine immunogenicity by targeting Ag to CD40-expressing dendritic cells. CD40.HIVRI.Env vaccine is fully humanized mAb fused to Cude CZM96 Env. We hypothesized that administration of different doses of CD40.HIVRI.Env vaccine adjuvanted with TLR3 Poly-ICLC Hiltonol® will be safe and immunogenic.

Methods: VR106 is a first-in-human phase I, placebo-controlled, dose-escalation trial (NCT04842682) in France and Switzerland. Twelve healthy volunteers were included per group (randomized 5:1 active vs. placebo) to receive either 0.3, 1, or 3 mg CD40.HIVRI.Env SC with Hiltonol® (1mg) at weeks (W) 0, 4, and 24. Safety, immunogenicity (anti-Env and anti V1/V2 IgG assessed by binding antibody multiplex assay, Neutralizing Abs (nAbs), IgG functionality, T-cell responses) were evaluated at W6, W26 and W48.

Results: Thirty-six volunteers were enrolled (mean 34 years, 64% male). Vaccine was safe and well tolerated. Two SAE were not related to vaccination. IgG response rates (RR) against vaccine-matched (np140.ZM96) and 6 mismatched gp140 and gp120 were 80-100% at W6 and 100% at W26 across groups. Magnitude of IgG responses (MFI) to Ags was high at all time points, peaked at W6 and/or W26 across groups (Figure) and remained flat or decreased slightly at W48. At W26, the BR to heterologous VTV2 Aps ranged from 60-100% (92TH02), 70-80% (CE0108), 50-100% (CaseA2) across groups. IgG3 anti-Env and
THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS IN STI-MEDIATED ENHANCED HIV INFECTION

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Background: Sexually transmitted infections (STIs) may affect the development and pathogenesis of other STIs, such as enhanced HIV infection. Understanding the mechanisms by which STIs enhance early events in HIV acquisition and replication would allow designing preventive strategies. Myeloid-derived suppressor cells (MDSCs) are a diverse population of immature myeloid cells that may exert immune-suppressive effects on other immune cells. Some of the cytokines produced during STIs have been involved in MDSC development. We therefore hypothesize that MDSCs expand in response to a pathogen in promoting immune suppression in cervix in patients with STIs. The enhancement of HIV infection after CT infection and the concomitant increase in MDSCs with a potentially suppressive phenotype, suggest a role for MDSCs in increasing the risk of secondary infection. Future efforts will include high-dimensional analyses of flow cytometry data and exploring the suppressive capacity of these cervical MDSCs.

Magnitude of IgG responses across groups and time points

Results: Cervical tissue was obtained from patients undergoing non-oncogenic cervical surgery. Chlamydia trachomatis or women with acute cervical BV were exposed to CT or GM-CSF+IL-6 enhanced subsequent HIV infection (n=7, p=0.02 and 0.003, respectively). Moreover, exposure to CT showed a trend towards an increase in the proportion of CD14+ MDSCs (p=0.07) and increased expression of suppressive mediators (e.g. PD-L1).

Conclusion: Distinct cytokine microenvironments and MDSC phenotypes may be involved based on the pathogen in promoting immune suppression in cervix in patients with STIs. The enhancement of HIV infection after CT infection and the concomitant increase in MDSCs with a potentially suppressive phenotype, suggest a role for MDSCs in increasing the risk of secondary infection. Future efforts will include high-dimensional analyses of flow cytometry data and exploring the suppressive capacity of these cervical MDSCs.

ENHANCED HIV INFECTION

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Background: BEAT2 (NCT03588715) tested combined peg-ifn-α2b with two broadly neutralizing antibodies (bnAbs) (3BNC117 and 10-1074) at antiretroviral treatment interruption (ATI) in 14 people living with HIV. Entry criteria included bnAb sensitivity by PhenoSense HIV mAb Assay (IC50 < 2.0 µg/ml, 3BNC117 and < 1.5 µg/ml, 10-1074). Primary clinical outcomes are presented elsewhere; here, we report the plasma rebound virus phenotype and evolution.

Methods: HIV-1 env single genome sequencing (SGS) was performed at rebound and longitudinally through ATI. Rebound lineage consensus Env was tested for neutralization sensitivity by TZM.bi assay and compared with PhenoSense results. Two participants withdrew during the study, 12 were analyzed.

Results: SGS of first detectable rebound virus (n=210 sequences) revealed a median of 1 (range 1-3) reactivating virus populations. Two participants rebounded with high levels of both bnAbs during the 26-week period of immunotherapy. Early rebound Env had complete resistance to 10-1074 (IC50 >10µg/ml), and increased resistance to 3BNC117 compared to entry criteria (median IC50 of 5.9 µg/ml). Eight participants rebounded after cessation of immunotherapy, between 26 and 30 weeks post-ATI, with waning 10-1074 and >50 weeks post-ATI as both bnAb levels fell to <5 µg/ml, retained sensitivity (>50 weeks post-ATI as both bnAb levels fell to <5 µg/ml, retained sensitivity to 10-1074 and 3BNC117). Rebound Env neutralization sensitivities largely agreed with PhenoSense cutoffs obtained from similar samples (100% concordance for 10-1074, 92% for 3BNC117). Rebound Env resistance to 3BNC117 correlated with time to rebound (r=0.82, p=0.013) and weakly correlated with concurrent 3BNC117 plasma levels (r=0.67, p=0.069). Longitudinal sequencing over ATI showed increasing env diversity via within-lineage evolution and addition of new rebound lineages, both of which led to further increases in resistance to 3BNC117 (median 2.5-fold increase in IC50).

Conclusion: Parallel analyses of rebound Env sensitivity to administered bnAbs using independent research and commercial assays gave similar results. Rebound Env lineages revealed a high frequency of resistance to both bnAbs (10-1074 > 3BNC117), which increased over the duration of ATI and correlated with time to rebound and diminishing bnAb levels.

TIGIT BLOCKADE RESTORES FUNCTIONAL NKG2C+ NK CELLS AND LIMITS SPREAD OF HIV-1 EX VIVO

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Background: TIGIT is an inhibitory receptor that is highly expressed on NK cells and inhibits antiviral activity, while its blockade has been shown to restore NK cell function and limit HIV spread in vivo. However, the in vivo effects of TIGIT blockade are not well understood.

Methods: We performed in vitro experiments to assess the effects of TIGIT blockade on NK cell function and HIV spread. We also conducted in vivo experiments in a rhesus macaque model of HIV infection to evaluate the efficacy of TIGIT blockade in limiting HIV spread.

Results: In vitro experiments showed that TIGIT blockade enhanced NK cell function and reduced HIV infection. In vivo experiments demonstrated that TIGIT blockade limited HIV spread in rhesus macaques.

Conclusion: TIGIT blockade restores functional NKG2C+ NK cells and limits spread of HIV-1 ex vivo.

326 BEAT2: PEG-IFN-ALPHA + 3BNC117 & 10-1074 REBOUND VIRUS PHENOTYPE AND EVOLUTION

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Background: BEAT2 (NCT03588715) tested combined peg-ifn-α2b with two broadly neutralizing antibodies (bnAbs) (3BNC117 and 10-1074) at antiretroviral treatment interruption (ATI) in 14 people living with HIV. Entry criteria included bnAb sensitivity by PhenoSense HIV mAb Assay (IC50 <2.0 µg/ml, 3BNC117 and <1.5 µg/ml, 10-1074).

Results: After ATI, 5/8 participants rebounded with high levels of both bnAbs during the 26-week period of immunotherapy. Early rebound Env had complete resistance to 10-1074 (IC50 >10µg/ml), and increased resistance to 3BNC117 compared to entry criteria (median IC50 of 5.9 µg/ml). Eight participants rebounded after cessation of immunotherapy, between 26 and 30 weeks post-ATI, with waning 10-1074 and >50 weeks post-ATI as both bnAb levels fell to <5 µg/ml, retained sensitivity to 10-1074 and 3BNC117. Rebound Env neutralization sensitivities largely agreed with PhenoSense cutoffs obtained from similar samples (100% concordance for 10-1074, 92% for 3BNC117). Rebound Env resistance to 3BNC117 correlated with time to rebound (r=0.82, p=0.013) and weakly correlated with concurrent 3BNC117 plasma levels (r=0.67, p=0.069). Longitudinal sequencing over ATI showed increasing env diversity via within-lineage evolution and addition of new rebound lineages, both of which led to further increases in resistance to 3BNC117 (median 2.5-fold increase in IC50).

Conclusion: Parallel analyses of rebound Env sensitivity to administered bnAbs using independent research and commercial assays gave similar results. Rebound Env lineages revealed a high frequency of resistance to both bnAbs (10-1074 > 3BNC117), which increased over the duration of ATI and correlated with time to rebound and diminishing bnAb levels.
BACKGROUND: Expression of TIGIT on Natural Killer (NK) cells from People living with HIV (PLWH) limits induction of adaptive NKG2C+ CD57+ cells and promotes a dysfunctional state after stimulation with dendritic cell (DC) immunotherapy. Here, we asked whether blockade of TIGIT enhances functional adaptive NKG2C+ NK cells in vitro and in vivo.

Methods: Monocyte-derived dendritic cells (DCs) from n=9 PLWH on ART and activated with nanoparticle-loaded Poly I:C (Nano-PIC) were cultured with autologous NK cells in the presence of anti-TIGIT or isotypic control mAbs. Reduction of proportions of HIV-1 p24+ cells CD4+ T cells in the presence of NK cells, Romipolin and Raltegravir was evaluated by FACS. In vivo efficacy of TIGIT blockade on NK cells was assessed by transplanting n=42 immunodeficient N6G mice with CD4+ T cells from the same PLWH alone (n=14) or with NK cells and Nano-PIC DC in combination with either anti-TIGIT (n=14) or isotypic mAbs (n=14) in three independent experiments. After 7 days, mice were sacrificed and proportions of circulating human CD45+ p24+ CD4+ T cells were analysed by FACS. In addition, histological distribution of p24+ infected cells in the spleen was analysed by confocal microscopy.

Results: Blockade of TIGIT combined with Nano-PIC DC enhanced the ability of NK cells from PLWH to significantly reduce by 70% the proportions of autologous HIV-1 p24 + CD4+ T cells ex vivo compared to cells receiving an isotypic mAb (p=0.0078). Importantly, i.p. administration of anti-TIGIT into N6G mice xenotransplanted with CD4+ T cells from PLWH and autologous NK cell and Nano-PIC DC immunotherapy led to a more significant reduction of p24+ cells (mean reduction 57%, p=0.0019) within human circulating CD4+ T cells compared to mice receiving only CD4+ T cells, in contrast to mice receiving isotypic control mAb (mean reduction 23.6%, p=0.0479). In addition, significantly lower areas of p24+/foci (Median 1,452 mm² vs 1,766 mm²; p=0.0078) as well as a trend to larger areas free of infection (Median 1,296.308 mm² vs 862.210 mm²) were detected in the spleen of mice injected with antiTIGIT compared to isotype mAbs. Interestingly, control of spread HIV-1 infection in spleen was associated with a reduction of CD57 expression (38% atTIGIT vs 63% isotype mAbs; p=0.0011) on adaptive NKG2C+ cells in this tissue.

Conclusion: Collectively, combination of DC immunotherapies and TIGIT blockade is a promising strategy to enhance functional NKG2C+ NK responses and limit HIV-1 replication in tissue in vivo.

Schematic Representation of in vitro and in vivo models used to study impact of modulation of TIGIT in functional restoration of NKG2C+ NK cells

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Background: An HIV vaccine that induces protective, broadly-neutralizing antibodies (bnAbs) prior to sexual debut is critical to eliminate the ~410,000 new infections annually that occur among youth aged 15 to 24 years worldwide, and provide lifelong immunity. Recent work has established that HIV-infected children develop bnAbs earlier and at a higher frequency than adults, suggesting that the infant immune landscape may be more amenable to the induction of bnAb B cell lineages by vaccination than that of adults. The goal of this study was to assess the ability of bnAb germline-targeting BG505 SOSIP trimers to induce precursor bnAb responses in early life.

Methods: Infant (n=5) and juvenile (n=4) rhesus macaques (RMs) received 3 immunizations of the germline-targeting BG505 GT1.1 SOSIP trimmer (50mg) with the 3M-052 SE adjuvant 6 weeks apart. All RMs were then boosted 12 weeks later with the wildtype BG505.664 SOSIP trimmer 3 times in 6-month intervals. Vaccine elicited antibody responses were monitored through 1.5 years.

Results: BG505 GT1.1 SOSIP trimmer immunization induced consistently higher magnitude vaccine antigen-specific IgG responses after each immunization in infants compared to juvenile RMs. BG505 SOSIP immunization also induced similar plasma tier 2 autologous virus neutralization responses in both age groups, which were maintained through 1.5 years. Notably, by week 80 three GT1.1 SOSIP-immunized infants exhibited a plasma neutralization signature reflective of CD4 binding site-specific (CD4bs) bnAb precursor development, while none of the juvenile RMs had developed this response. Electron-microscopy based epitope mapping of polyclonal plasma confirmed the presence of CD4bs-targeting antibodies in the majority of immunized infant RMs.

Conclusion: Our data demonstrates that sequential immunization of infant RMs with germline-targeting and wildtype BG505 SOSIP trimers can induce high serum neutralizing antibody titers and CD4bs bnAb precursors, while these responses were not detectable in vaccinated juvenile RMs. Our results support observations in human studies suggesting that the infant immune environment is better suited for induction of plasma HIV bnAb responses.

In vitro culture of primary cells from PLWH

In vivo humanized N6G mouse model

7 days FACs p24 Nkis

329 DYNAMICS AND ROLE OF TOX+ TCF1+ CD39+ CD8 T CELLS IN LYMPHOID TISSUE IN SIV INFECTION

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Background: CD8 T-cell responses to HIV/SIV infection critically contribute to control of viremia, but lymph node (LN) CD8 T-cells have been shown to be phenotypically and functionally distinct from those in blood.

Methods: To characterize the unique populations in LN, we obtained LN samples from rhesus macaques during early chronic SIV infection (D42 post-infection) and performed flow cytometry, scRNA-seq and imaging to explore the dynamics of LN CD8 T-cells expressing Tox, TCF1 and CD39, three well-described markers delineating exhausted, stem-like and terminally-differentiated populations.

Results: Tox is upregulated after SIV infection and primarily expressed in PD-1+ and TIGIT+ cells. Notably, we observed high expression of Tox on a previously undescribed TCF1+CD39+ population, distinct from the known TCF1+CD39+ stem-like and TCF1+CD39+ effector/exhausted CD8 T-cells, that significantly expands after infection. These TCF1+CD39+ cells expressed inhibitory receptors, intermediate levels of Ki-67, and are uniquely high in GzMB but low in GzMK, a profile consistent with a precursor effector cell. After SIV peptide stimulation, TCF1+CD39+ cells degranulated at similar levels to TCF1+CD39+ cells but produce less IFNγ. scRNA-seq of SIV-specific CD8 T-cells revealed an intermediate profile of TCF1+CD39+ cells between the stem-like TCF1+CD39+ and the differentiated TCF1+CD39+ cells. Importantly, a higher frequency of both Tox+ (p<0.0001) and CD39+ (p=0.0003) CD8 T-cells in LN at d42 p.i. was strongly associated with lower plasma viremia, lower levels of cell-associated SIV-DNA (Tox p=0.0007; TCF1+CD39+ p=0.01) and better CD4 preservation (Tox p=0.02; TCF1+CD39+ p=0.065). After more than 1 year of ART, the level of TCF1+CD39+ LN CD8 T-cells is associated with lower frequency of CD4 T-cells harboring intact SIV-DNA (IPDA). Investigations into potential mechanisms contributing to viral control revealed that TCF1+CD39+ cells expressed elevated levels of CXCR5 compared to traditional effector cells, and expression of CXCR5 within TCF1+CD39+ cells was associated with lower plasma viremia. Imaging analysis confirmed increased presence of TCF1+Tox+CD39+ T-cells within B cell follicles compared to TCF1- and TCF1+Tox- cells.

Conclusion: Overall, these data are consistent with a unique precursor effector CD8 T-cell population that expands in LN after SIV infection, have a better ability to access the LN BCF, and is associated with increased viral control and reduced disease progression.
330 SPATIAL MAPPING OF SIV-INFECTED RHESUS MACAQUE LYMPH NODE ENVIRONMENT DURING REBOUND

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Background: Lymphoid tissues are important sites of the HIV-1 reservoir that contribute to viral rebound after analytical treatment interruption (ATI). We hypothesize that different immune cell subsets exhibit distinct immune signatures during early versus late viral rebound. Understanding these immune signatures and their respective immune cell subsets throughout viral rebound provides mechanisms for immune control.

Methods: From 2 female rhesus macaques intravaginally infected with SIVsm-cr1 and treated with suppressive antiretroviral therapy (ART) for 6 months from 4 days post-challenge, we obtained 2 mesenteric lymph nodes each at 4 days and 24 days after ATI. These lymph nodes were SIV+ by targeted PET-CT. To resolve lymphoid cell heterogeneity and their respective immune responses, particularly in the context of spatial interactions, we applied deterministic barcoding in tissues (DBiT-seq) to profile transcriptomes from 10 μm x 10 μm pixels. This state-of-the-art platform allows the closest estimate of single-cell transcriptomes at a genome-wide scale compared with currently available platforms. We used machine learning algorithms to assign cell type identities to pixels, created spatial maps overlaying the pixels on tissue images, and determined gene set enrichment in early versus late viral rebound with GSEA.

Results: To identify early and late viral rebound immune signatures, we profiled 9822 10 μm x 10 μm pixels. We captured a median of 265 RNA reads and 163 genes per pixel. At 4 days after ATI, we found enrichment in gene signatures for Fregs (GSE37605, leading edge genes: IGBP2, TAGLN, FHL1, MGP, HSPB1), cytotoxic macrophages (GSE26912: LPL, FASN, RRA5, S100A1, SPARC; GSE22935: MYL9, GSN, LPL, FABP4, WDFY3), T cell subsets (GSE21379: FHL1, DGAT2, S100A1, LAMB2, AKAP12), and expanding CD4+ T cells (GSE32533: HSPB6, IFG2, MLXIP, CRIP2, NEOM; GSE9316: GSN, TNFR2, VCL, LDB2). At 24 days after ATI, we found upregulated gene signatures of stimulated neutrophils (GSE2405: ACTG1, CD3, COROA, CUL1, RPL3) and vaccine responses to Leishmania (Troy: CXCL9, CD74, CTHS, LYZ, C3), VEEV (Erwin Cohen: L127, IFIT2, RPL5, IFI6, CD52), influenza (Nakaya: CTHS, IFIT3, SW, PSAP, CD84, LCK), and HIV-1 (Zak MRKAD3: C1Q8, IFIT2, IFI6, TRIMFES18, TCIRG1).

Conclusion: We identified CD4+ T cell and macrophage signature early and immune effector cell responses later during viral rebound in lymph nodes. Further spatial analysis and validation could provide targets to improve immune control and function during rebound.

331 LYMPH NODE IMMUNE MICROENVIRONMENT FOLLOWING ART INITIATED DURING ACUTE HIV INFECTION

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Background: Persistence HIV reservoirs in lymphoid tissues is one of the main barriers to cure. We and others have shown that follicles within secondary lymphoid tissues are a major site of HIV persistence during antiretroviral therapy (ART).

Methods: We used spatial transcriptomics and several imaging approaches to spatially define cellular subsets and molecular pathways associated with HIV persistence in LN. Study participants included 45 women (16 to 24 years old) who initiated therapy in Fiebig stage I-V, and 10 age matched women who started therapy after Fiebig V. Exclusionary LNs were obtained after a year or more on uninterrupted ART.

Results: Using multicolor immunofluorescence staining and in situ hybridization we were able to detect HIV Gag24 protein and Gag-pol RNA in GC T follicular helper cells (GCTFH) and macrophages in 18 of 20 individuals studied. GCs had greater amounts of HIV Gag p24 protein and Gag-pol RNA compared to extrafollicular areas (p<0.0001). Very early ART was associated with more functional (proliferative) HIV-specific CD8+ T cells compared to late treated donors (p=0.002). However, most of the CD8+ T cells were spatially segregated from HIV harbouring GCTFH and macrophages. The few CD8+ T cells within GCs lacked expression of cytolytic molecules granzyme B and perforin. Spatial transcriptomic analysis revealed lower expression of adaptive immune response genes in GCs harbouring HIV antigens relative to adjacent GCs without HIV antigens. Inversely, immunoregulatory genes namely, FasP3 and Tbet (tbx21) were upregulated in HIV positive GCs relative to HIV negative GCs.

Conclusion: We show that persistent HIV transcription in GCs generates spatially defined immunosuppressive microenvironment. These data suggest that reversing immunosuppressive environment within HIV infected GCs is critical to the development of CD8+ T cell-mediated strategies aimed at eliminating HIV infection in lymphoid tissue during ART.

332 DEVELOPMENT OF A LYMPHOID ORGAN-CHIP TO EVALUATE COVID VACCINE BOOSTING STRATEGIES

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Background: Secondary lymphoid organs provide the adequate microenvironment for the development of antigen (Ag)-specific immune responses. The tight collaboration between CD4+ T cells and B cells in germinal centers is crucial to shape B cell fate and optimize antibody maturation. Dissecting these immune interactions remains challenging in humans, and animal models do not always recapitulate human physiology. To address this issue, we developed an in vitro 3D model of a human lymphoid organ. The model relies on a microfluidic device, enabling primary human cells to self-organize in an extracellular matrix (ECM) under continuous fluid perfusion. We applied this Lymphoid Organ-Chip (LO chip) system to the analysis of B cell recall responses to SARS-CoV-2 antigens.

Methods: We used a two-channel microfluidic Chip S1 from Emulate, where the top channel is perfused with antigen (spike protein or SARS-CoV-2 mRNA vaccine), while the bottom channel contains PBMC (n = 14 independent donors) seeded at high-density in a collagen-based ECM. Immune cell division and cluster formation were monitored by confocal imaging, plasmoning differentiation and spike-specific B cell amplification by flow cytometry, antibody secretion by a cell-based binding assay (S-flow).

Results: Chip perfusion with the SARS-CoV-2 spike protein for 6 days resulted in the induction CD83+CD27+ plasmablast maturation compared to an irrelevant BSA protein (p < 0.0001). Using fluorescent spike as a probe, we observed a strong amplification of spike-specific B cell from 3.7 to 140-fold increase. In line with this rapid memory B cell response, spike-specific antibodies production could be detected as early as day 6 of culture. Spike perfusion also induced CD4+ T cell activation (CD38+ ICOS+), which correlated with the level of B cell maturation. The magnitude of specific B cell amplification in the LO chip was higher than in 2D and 3D static cultures at day 6, showing the added value of 3D perfused culture for the induction of recall responses. Interestingly, the perfusion of mRNA-based SARS-CoV-2 vaccines also led to strong B cell maturation and specific B cell amplification, indicating that mRNA-derived spike could be expressed and efficiently presented in the LO chip.

Conclusion: We developed a versatile Lymphoid Organ-Chip model suitable for the rapid evaluation of B cell recall responses. The model is responsive to protein and mRNA-encoded antigens, highlighting its potential in the evaluation of SARS-CoV-2 vaccine boosting strategies.

333 AGING IMPAIRS HIV RESPONSES IN NEUTROPHILS FROM BLOOD AND THE FEMALE GENITAL TRACT

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Background: Women acquire HIV through sexual transmission, with HIV incidence increasing in older women (>50 years). However, low transmission rates per sexual act indicate local immune protection against HIV acquisition at the main portal of entry. We demonstrated that genital neutrophils respond to HIV stimulation with Neutrophil Extracellular Trap (NET)-release to inactivate the virus. However, the mechanisms involved in HIV recognition, molecular events that lead to NET-release, and how aging affects this protective mechanism are unknown.

Methods: Human neutrophils were purified from blood (n=36) and genital tract tissues (n=45) after digestion of hysterectomy samples (endometrium, ectocervix and endocervix). Neutrophils were stimulated in vitro with...
GFP-labeled HIV to induce NET-release and quantified by time-lapse imaging and confocal microscopy. To study HIV-recognition and signaling mechanisms, prior to HIV stimulation, neutrophils were incubated with endosomal TLR7/8 inhibitors; Rhod-AM to stain cytosolic calcium; and Annexin V dye to assess cell death.

**Results:** Early NET-release (15min) was reduced in post- compared to pre-menopausal women (p=0.05) and progressively declined with aging (r=-0.6; p< 0.01). In pre-menopausal neutrophils, HIV stimulation significantly increased cytosolic calcium levels immediately after stimulation (p< 0.01), but HIV-induced calcium response declined with age (r=-0.6; p=0.02). In post-menopausal women, NET release was preferentially triggered by endosomal TLR recognition of HIV-NA and TLR8 and TLR7 inhibition reduced early (15min) and late (2h) NET-release respectively. Reduced NET-release in post-menopausal women was not due to increased cell death. However, we identified a subset of NETs that incorporated Annexin V, were preferentially released at late time points by blood neutrophils, but not tissue neutrophils, and uniquely incorporated lactoferrin, with known proinflammatory properties.

**Conclusion:** HIV-induced NET-release is delayed and progressively declines in post-menopausal neutrophils from blood and genital tissues. In younger women, HIV triggers rapid cytosolic calcium increase, but this response is compromised with aging, leading to preferential endosomal TLR7/8 induced NET-release in post-menopausal women. Importantly, we identified differential responses in blood and tissue, with blood neutrophils more prone to release proinflammatory NETs, highlighting the need to study genital neutrophils to develop prevention strategies.

### 334 RAPID EXPANSION OF MEMORY NK CELLS IN ACUTE HIV INFECTION WHICH PERSIST DESPITE ART

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**Background:** HIV infection is associated with NK cell dysfunction including expansion of memory NK cells with heightened inflammatory and cytotoxic function which persist despite antiretroviral therapy (ART). We investigated the timing of NK cell activation following acute HIV infection (AHI) and explored the impact of early ART initiation on these parameters.

**Methods:** PBMC and plasma samples were obtained from people with acute HIV (PWH) in the RV254 cohort who initiated ART during Fiebig stages I, II, III or IV/V (n=8-10 in each). Immunophenotyping was performed at AHI (pre-ART) and 4, 12 and 48 weeks after ART-initiation. IgG antibodies to cytomegalovirus (CMV) antigens were assessed by ELISA. Participants were all men who have sex with men (MSM), median age 26.0 (IQR: 22.8-30.0) years and all achieved viral suppression after 48 weeks of ART. Samples from people without HIV (PWOH) enrolled in the RV304 study (n=10 MSM, median age 27.0 [23.8 – 38.5]) were analyzed for comparison.

**Results:** Proportions of memory NK cells (defined by absence of the Fcg receptor signal transduction chain FcR6Ig) were significantly expanded in PWAH in all Fiebig stages pre-ART (pooled median 66% vs 25% for controls, p< 0.001). Memory NK cells were not different between Fiebig stages and were not altered by ART. Plasma antibodies to the immediate early CMV protein were elevated in PWAH in Fiebig III and IV/V (p = 0.03 for both), suggesting more frequent CMV reactivations secondary to HIV-induced immunosuppression. Proportions of memory NK cells were significantly negatively associated with HIV DNA in PBMC at diagnosis (p=0.04) and trending towards an association after 48 weeks of ART (p=0.08). NK cells were activated in PWAH in all Fiebig stages including stage I (CD69+; p=0.02 and HLA-DR+ /CD83; p=0.04), whilst activation of CD4 and CD8 T cell became evident at Fiebig stages II or after. Suppressive ART significantly reversed T cell activation to levels comparable to PWOH after 48 weeks whilst proportions of activated HLA-DR+ /CD83 + NK cells remained elevated irrespective of when ART was initiated.

**Conclusion:** NK cell activation and expansion of memory NK cells occurs very early during AHI, prior to T cell activation and CMV reactivation, and is not reversed by early ART. The association of memory NK cells with lower HIV DNA levels may suggest a role in HIV control. These findings have implications for the long-term morbidity of PWH on ART.

### 335 HIERARCHICAL CLUSTERING SHOWS B-CELL PERTURBATIONS INDEPENDENT OF HIV-2 VIRAEMIA


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**Background:** Time to AIDS in HIV-2 infection is approximately twice as long compared to in HIV-1 infection. Still, and despite reduced viraemia, HIV-2 infected individuals display signs of chronic immune activation. In HIV-1 infected individuals, the expansion of hyperactivated B-cells, characterized by the expression of the transcription factor T-bet, is driven by continuous antigen exposure. However, the contribution of viraemia to B-cell perturbations in HIV-2 infected individuals remains largely unexplored. Here we set out to determine if B-cell hyperactivation is viraemia dependent during HIV-2 infection, as it has been described for HIV-1 infection.

**Methods:** We used polychromatic flow cytometry to immunophenotype B-cells from HIV-1 infected (viraemic (n=8) and successfully ART treated (n=7) individuals), HIV-2 infected (viraemic (n=8) and treatment naive (n=12) individuals), and HIV seronegative (n=25) individuals from Guinea-Bissau. We performed consensus hierarchical cluster analysis on the flow cytometry data, using the FlowSOM R packages, to define the impact of HIV-1 or HIV-2 infections on the expansion or depletion of B-cell populations. Plasma proteins were quantified using an Olink Proteomics panel. Frequencies of identified B-cell populations were correlated with concentrations of Th1 associated proinflammatory cytokines.

**Results:** We observed increased frequencies of clusters containing hyperactivated T-bet<sup>++</sup>CD95<sup>++</sup> and proliferating CD95<sup>++</sup>T-bet<sup>++</sup>CD27<sup>++</sup>B-memory B-cells in viraemic HIV-1 (p< 0.001 and p< 0.001, respectively), viraemic HIV-2 (p< 0.01 and p=0.014, respectively), and in treatment naive aviremic HIV-2 (p=0.004 and p=0.020, respectively) infected individuals compared to seronegative individuals. In contrast, these expansions were not observed in successfully treated HIV-1 infected individuals. Finally, the frequency of the hyperactivated Tbet<sup>++</sup>B-memory B-cells showed a moderate to strong correlation with plasma concentrations of proinflammatory Th1-associated cytokines, most prominently TNF-α (r=0.686; p=0.001), IFN-γ (r=0.530; p=0.020), CXCL10 (r=0.619; p=0.005) and CXCL10 (r=0.674; p=0.002) in HIV-2 infected individuals.

**Conclusion:** In contrast to successfully ART treated HIV-1 infected individuals; treatment naive aviremic HIV-2 infected individuals showed B-cell perturbations. Our data therefore suggest that aviremic HIV-2 infected individuals are likely to benefit from antiretroviral treatment.

### 336 EARLY ART TREATMENT PREVENTS B CELL LOSS AND DYSFUNCTION IN PEOPLE LIVING WITH HIV

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**Background:** PEOPLE LIVING WITH HIV long-term morbidity of PWH on ART.

**Methods:** Specimens from participants in the RV217 Early Capture HIV Cohort (ECHO) and RV254 Acute HIV Infection (AHF) study were used to assess B cell changes in PWH who are therapy-naive or treated during early AHI, respectively. Flow cytometry was employed to determine B cell counts and phenotypes in both peripheral blood mononuclear cells (PBMC) and mucosal mononuclear cells...
**Results:** PWH have reduced peripheral B cell counts at diagnosis of AHI; however, administration of early ART resulted in higher peripheral B cell counts and frequencies as early as 2 weeks after ART initiation compared to untreated PWH (p<0.05). PWH treated during Feibig stage II or III showed significant increases in peripheral B cell counts between week 0 and week 2 post ART (p<0.01), whereas PWH treated during Feibig stage I maintained similar B cell counts at both time points (p>0.05). In the absence of ART, the frequency of peripheral memory B cells decreased and plasmablast populations increased 2 weeks after infection (p<0.01) compared to uninfected time points. However early administration of ART prevented these changes (p>0.05). Conversely, compared to healthy donors, frequencies of mucosal memory cells were expanded at HIV diagnosis during AHI (week 0) and then declined after ART administration. However, these memory B cell changes were not detected in the periphery (see Figure).

**Conclusion:** Early ART administration maintained similar peripheral B cell phenotypes compared to healthy donors whereas later treatment resulted in increased B cell activation and differentiation, likely due to increased antigen load. However, B cell population changes in the sigmoid mucosa were still detected despite early ART administration, suggesting early B cell responses to HIV in mucosal compartments. The frequency of memory B cells from the periphery (A) or sigmoid biopsies (B) of uninfected (HIV Neg) or ART treated PWH (Week 0–96) was determined by flow cytometry. P-values were calculated using Mann-Whitney.

### 337 NK CELL BIOMARKERS THAT PREDICT TIME-TO-REBOUND IN HIV+ INDIVIDUALS UNDERGOING ATI

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**Background:** Most people with HIV exhibit viral rebound within weeks of analytical treatment interruption (ATI), but some can control replication for prolonged periods of time. The mechanisms mediating control upon ATI remain poorly understood, and may involve Natural Killer (NK) cells, potent antiviral immune effectors. The objective of this study was to use CyTOF to identify NK cell features that can predict time-to-rebound upon ATI.

**Methods:** We analyzed samples from two ATI cohorts: the REDUC cohort, which consisted of 17 participants that received therapeutic vaccination (Vac4ck) with the HDAC inhibitor Romidespin prior to ATI, and the ACTG A5345 cohort, which consisted of 33 chronic and 12 acute-treated participants that underwent ATI in the absence of any intervention. We phenotyped pre-ATI PBMCs using an NK cell CyTOF panel that includes markers of differentiation and activation states, homing receptors, inhibitory and activating receptors, and intracellular factors. We performed unsupervised clustering analyses to define NK cell clusters, optimized the number of clusters with a leave-one-out cross-validation model, and looked for associations with time-to-rebound, with HIV reservoir size as determined by IPDA, and with time of treatment initiation (chronic vs. acute).

**Results:** Clustering analyses for the REDUC cohort revealed a cluster that was significantly (p<0.05) associated with increased time-to-rebound: an individual with 2.1 times the average number of cells belonging to this cluster rebounds on average 3 days later. The NK cells in this cluster were atypical in that they were CD56-CD16+ and exhibited a memory signature. Clustering analyses for the ACTG A5345 cohort revealed that individuals with a lower frequency of intact provirus were significantly (p<0.01) associated with a different cluster of CD56+CD16+ NK cells expressing high levels of the inhibitory receptor Siglec-7 associated with functional NK responses. Acute-treated participants were also more likely to have NK cells that expressed high levels of the memory NK cell antigens CXCR6 (p<0.01) and CD49a (p=0.06).

**Conclusion:** As CD56-CD16+ NK cells have been associated with bnAb production, these results suggest a potential role of NK cells and HIV-specific antibodies in control of viral replication. Siglec-7+ NK cells may temporarily control viral rebound during ATI. The findings with CXCR6 and CD49a implicate a role for memory NK cells in the preserved immune responses found in acute-treated individuals.

### 338 CHARACTERIZATION OF TISSUE-RESIDENT NK CELLS IN A TISSUE MODEL OF HIV INFECTION

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¹HIV Translational Research

**Background:** NK cells are innate immune cells with important functions in controlling HIV infection. However, their role in the main tissues where HIV persists, such as the secondary lymphoid tissue and the gastrointestinal tract, is largely undefined. Here, we have characterized NK cell present in relevant tissues, particularly their memory-like and residency properties, and assessed their functionality in killing HIV-infected cells in a tissue model of HIV infection.

**Methods:** We obtained tissue resections from gut and tonsils from uninfected donors. We determined by flow cytometry the expression of the memory marker NKG2C, the residency markers CD49a, CD69, and CD103, and KIRs: (KIR2DL1, 51, 52, L3, and S2). Natural cytotoxicity was evaluated by the expression of CD107a and IFN-y after co-culturing disaggregated tissue cells with the HLA-negative K562 cell line (tonsil n=11, gut n=7). Last, using the tonsil explant model (n=16), the NK phenotype and natural cytotoxicity were characterized by flow cytometry after 5-7 days of HIV-BAL ex vivo infection.

**Results:** Gut tissue had higher proportions of CD56+ NK cells (1.8%) in comparison with tonsils (0.38%). The distribution of the two main NK subsets was similar, showing a predominant population of CD56brightCD16- and CD56dimCD16+. However, their minority counterpart (CD56+CD16-) was the subset more frequently expressing the residency markers CD49a and CD103, and KIRs. NK cells had higher expression of the residency markers CD69 and CD103; however, they showed a reduced stimulatory potential compared with tonsils. In general, tonsil CD16+ NK cells expressing residency markers correlates with decreased levels of HIV in ex vivo infection. Furthermore, we detected two main tonsil populations associated with significant lower levels of HIV: the CD56brightCD16-CD103+ subset (r=-0.54, p=0.014), and the CD56dimCD16+CD69+KIR-+ cells (r=-0.61, p<0.01), being the last significantly expanded in the infected culture (p<0.01). Importantly, both subpopulations showed high basal production of CD107a and IFN-y and increased functionality after K562 stimulation.

**Conclusion:** Relevant anatomic sites for HIV present different NK subset distribution and functional capacity. Our research indicates that higher numbers of cytotoxic NK-resident cells might have an important role in HIV control in lymphoid tissue. Hence, strategies aimed at expanding tissue-resident NKs may lead to the development of targeted therapies directed to the main sites of HIV persistence.

### 339 LOW-DENSITY GRANULOCYTES DISPLAY IMMATURE CELLS WITH PRIMED PHENOTYPE IN PLWH

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**Background:** While a protective role of neutrophil extracellular traps (NET) in limiting HIV spread to susceptible cells has been documented, there is comparatively little insight into whether NET formation may also be harmful in people living with HIV (PLWH) as a potential proinflammatory driver of HIV-associated comorbidities. To gain insight into this issue, we assessed the
frequency and characteristics of low-density granulocyes (LDGs), a subset of neutrophils and their NET forming capacity in PLWH on antiretroviral therapy (ART) and in HIV sero-negative (HIV-) individuals.

Methods: Utilizing banked PBMCs from the Hawaii Aging with HIV-Cardiovascular (HAIHC-CV) study, we identified and quantified circulating LDGs (CD14- CD16+ CD15+) by flow cytometry. We compared CD84 maturation and activation, assessing CD10 expression and mean fluorescence intensity (MFI) of cell surface markers, CD11b, CD66b, and MPO per LDG, respectively. NET forming LDGs (MPO+ H3Cit+) were analyzed and plasma levels of NET (H3Cit and cDNA) were measured to detect NET components in blood. We also measured circulating inflammatory (IL-6 and CRP) and prothrombotic markers (fibrinogen and d-dimer) to assess their relationship to plasma NET levels.

Results: A total of 141 subjects, (HIV+: n=88 and HIV-: n=53) were included in this study. The median (IQR) age was 49 (45.0-57.0) years (87.8% male) in HIV+ individuals and 55 (47.5-60.0) years (84.9% male) in the HIV- individuals. Most participant (89.0%) had undetectable HIV RNA (less than 50 copies/mL) and the median CD4+ T cell count was 469 (294.5-605.5) cells/mm3. The number of LDGs per participant (89.0%) had undetectable HIV RNA (less than 50 copies/mL) and the median CD4+ T cell count was 469 (294.5-605.5) cells/mm3. The number of LDGs was significantly decreased in HIV+ (p = 0.03) compared to HIV- individuals (495.7 (262.5-1585)), 1038 (442.3-3774), respectively). We observed significantly higher NET forming capacity in HIV+LDG compared to HIV- LDG. Moreover, HIV+ LDG displayed immature CD10- neutrophils, but had heightened expression of CD66b and CD11b. Increased H3Cit levels (p < 0.001) were found in HIV+ individuals and these levels were positively associated with inflammatory and prothrombotic markers.

Conclusion: We found that LDGs from HIV+ individuals on ART display an immature and activated phenotype, and may exert proinflammatory effects. Plasma NET levels correlate with blood markers for inflammation and coagulation, which may yield insights into the pathologic role of LDGs at least in part mediated through NET formation in PLWH.

Comparison of LDG counts and the proportion of NET forming LDGs in HIV+ and HIV- individuals

Figure 1. Comparison of LDG counts and the proportion of NET forming LDGs in HIV+ and HIV- individuals. Data points are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, compared to HIV- controls. N = 88 HIV+ and 53 HIV- individuals. A) LDG count, B) NET forming LDGs, C) MPO+ H3Cit+, D) MPO+ H3Cit+ and NET forming LDGs. Spearman's correlation test was used for statistical analysis.

340 NK CELLS ARE CRITICAL FOR IN VIVO CONTROL OF SARS-CoV-2 REPLICATION AND DISEASE

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Background: Natural killer (NK) cells play a critical role in control of viral infections. However, empirical evidence thus far has been unclear on the role of NK cells in pathogenesis and control of SARS-CoV-2 infection with some research suggesting NK cell accumulation as beneficial while others indicate it as deleterious. To address this crucial deficit in understanding, we employed a non-human primate infection model with a validated experimental NK cell depletion technique.

Methods: A total of 12 experimentally naïve (75% female) cynomolgus macaques (CM) of Cambodian origin were used in this study. Six CM were NK cell-depleted using an anti-IL-15 neutralizing antibody, while six controls received placebo, prior to intranasal and intratracheal challenge with the SARS-CoV-2 Delta variant at a TCID50 of 1X10^5. The cohort was monitored for five weeks with scheduled blood, colorectal (CR) biopsies, and lymph node (LN) collections. Total envelope and sub-genomic viral loads (VL) were measured in the nasal cavity, throat, and bronchoalveolar lavage (BAL). 23-color flow cytometry, pathology, and 27-plex inflammatory analyte Luminex analyses were conducted. Statistical tests used were Mann-Whitney U and Spearman's Correlation.

Results: Control CM exhibited an increase in the frequency of circulating NK cells, reaching a peak at 10 days post-infection (DPI) and returning to baseline by 22DPI. Simultaneously, NK cells expressing activation and tissue retention marker, CD69, also significantly increased. G7toxic NK cells were positively associated with VL (r = 0.66; p = 0.02), suggestive of a virus-induced mobilization.

Total experimental NK cell ablation was verified in blood, CR, and LN of NK cell-depleted CM, which had higher VL compared to controls in all tissues evaluated, reaching significance at 10DPI (p = 0.01) and demonstrated a longer duration of viremia. Although Luminex measures were similar in plasma, BAL samples from NK cell-depleted CM had universally higher concentrations of inflammatory mediators, most notably a 25-fold higher concentration of IFN-α compared to controls. Lung pathology scores were also higher in NK cell-depleted CM with increased evidence of fibrosis, synctyia, pneumocyte hyperplasia, and endothelitis.

Conclusion: Overall, we find significant and conclusive evidence for NK cell-mediated control of SARS-CoV-2 virus replication and disease pathology. These data suggest adjunct therapies for infection could largely benefit from NK cell-targeted approaches.

341 SIGLEC-9 RESTRAINTS ANTIBODY-DEPENDENT NK CELL CYTOTOXICITY AGAINST SARS-CoV-2

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Background: SARS-CoV-2 infection alters the immunological profiles of natural killer (NK) cells. However, whether NK anti-viral functions (direct cytotoxicity and/or antibody-dependent cell cytotoxicity (ADCC)) are impaired during severe COVID-19 and what host factors modulate these functions remain unclear.

Methods: Using functional assays, we examined the ability of NK cells from SARS-CoV-2 negative controls (n=12), mild COVID-19 patients (n=26), and hospitalized COVID-19 patients (n=41) to elicit direct cytotoxicity and ADCC (NK degranulation by flow) against cells expressing SARS-CoV-2 antigens. SARS-CoV-2 N antigen plasma load was measured using an ultra-sensitive Simoa assay. We also phenotypically characterized the baseline expression of NK activating (CD16 and NKG2C), maturation (CD57), and inhibitory (NKG2A and the glyco-immune restriction on NK cytotoxicity can further improve NK immune surveillance and function during severe COVID-19 and what host factors modulate these functions remain unclear.

Results: Our data suggest that Siglec-9+ NK cells express high levels of NKG2A, than Siglec-9- NK cells (P≤0.03). These data are consistent with the concept that the NK cell subpopulation expressing Siglec-9 is highly mature phenotype with higher expression of CD16, CD57, and NKG2C, maturation (CD57), and inhibitory (NKG2A and the glyco-immune

Conclusion: Overall, we find significant and conclusive evidence for NK cell-mediated control of SARS-CoV-2 virus replication and disease pathology. These data suggest adjunct therapies for infection could largely benefit from NK cell-targeted approaches.

342 RESTRAINTS ANTIBODY-DEPENDENT NK CELL CYTOTOXICITY AGAINST SARS-CoV-2

1Wistar Institute, Philadelphia, PA, USA, 2Rush University, Chicago, IL, USA, 3University of Vermont, Burlington, VT, USA, 4Rush University Medical Center, Chicago, IL, USA, 5University of Vermont, Burlington, VT, USA, 6GeneSight Life Science, Fort Washington, PA, USA

Background: SARS-CoV-2 infection alters the immunological profiles of natural killer (NK) cells. However, whether NK anti-viral functions (direct cytotoxicity and/or antibody-dependent cell cytotoxicity (ADCC)) are impaired during severe COVID-19 and what host factors modulate these functions remain unclear.

Methods: Using functional assays, we examined the ability of NK cells from SARS-CoV-2 negative controls (n=12), mild COVID-19 patients (n=26), and hospitalized COVID-19 patients (n=41) to elicit direct cytotoxicity and ADCC (NK degranulation by flow) against cells expressing SARS-CoV-2 antigens. SARS-CoV-2 N antigen plasma load was measured using an ultra-sensitive Simoa assay. We also phenotypically characterized the baseline expression of NK activating (CD16 and NKG2C), maturation (CD57), and inhibitory (NKG2A and the glyco-immune

Conclusion: Overall, we find significant and conclusive evidence for NK cell-mediated control of SARS-CoV-2 virus replication and disease pathology. These data suggest adjunct therapies for infection could largely benefit from NK cell-targeted approaches.
pDC sensing of SARS-CoV-2 VDC-infected cells suggests that some VOCs might manipulate the interactions of pDCs with infected cells to limit IFN-I responses.

### 343 POLYFUNCTIONAL SARS-CoV-2-SPECIFIC T CELLS PERSIST IN TISSUE OF COVID-19 CONVALESCENTS

**Kyrilia C. Young**, Ashley F. George, Matthew McGregor, Xiaoyu Luo, Rebecca Marquez, Kailin Yin, Tongcui Ma, Trimble Spitzer, Nadia R. Roan

**Background:** T cells play an essential role in SARS-CoV-2 immunity, including in defense against severe COVID-19. However, most studies analyzing SARS-CoV-2-specific T cells have been limited to analysis of blood. Furthermore, the role of T cells in SARS-CoV-2 immunity in pregnant women, which are at disproportionately higher risk of severe COVID-19, is poorly understood.

**Methods:** Here, we quantitated and deep phenotyped SARS-CoV-2-specific T cells from convalescent women (n=12) that had mild (non-hospitalized) COVID-19 during pregnancy. Endometrial, maternal blood, and fetal cord blood specimens were procured at term, which ranged from 3 days to 5 months post-infection. SARS-CoV-2-specific T cells were deeply analyzed by CyTOF using a tailored phenotyping panel designed to assess the effector functions, differentiation states, and homing properties of the cells.

**Results:** SARS-CoV-2-specific T cells were more abundant in the endometrium than in maternal or fetal cord blood. In a particularly striking example, in one donor sampled 5 months after infection, SARS-CoV-2-specific CD8+ T cells comprised 4.8% of total endometrial CD8+ T cells, while it only reached 1.4% in blood. Endometrial SARS-CoV-2-specific T cells were more frequently of the memory phenotype relative to their counterparts in maternal and fetal cord blood, which harbored higher frequencies of naive T cells. Relative to their counterparts in blood, endometrial SARS-CoV-2-specific T cells exhibited unique polyfunctional features, including preferential expression of the T resident memory marker CD69, inflammatory tissue-homing receptor CXCR4, and the activation marker 4-1BB. Endometrial T cells were highly polyfunctional, and could secrete IFNγ, TNFα, MIP1β, IL2, and/or IL4 in response to spike peptide stimulation. By contrast, their counterparts in blood preferentially produced the cytolytic effectors perforin and granzyme B.

**Conclusion:** Polyfunctional SARS-CoV-2-specific T cells primed by prior exposure to the virus are abundant and persist in endometrial tissue for months after infection. These cells exhibit unique polyfunctional features including preferential expression of select chemokine receptors and activation molecules. Compared to their blood counterparts, the effector functions of these cells are more cytokine-driven and less cytolytic. The long-term persistence of these cells in the endometrium may help protect future pregnancies from SARS-CoV-2 re-infection.

### 344 HIGH RNAemia ASSOCIATES WITH SKewed T-CELL RESPONSE IN PLWH HOSPITALIZED FOR COVID-19

**Matteo Augello,** Valeria Boni, Roberta Rovito, Camilla Tincati, Silvia Bianchi, Lucia Taramasso, Antonio Di Biagio, Annapaoleta Callegari, Franco Maggiolo, Elisa Borghi, Antonella D’Arminio Monforte, Giulia C. Marchetti

**University of Milan, Milan, Italy, 2San Martino Policlinico Hospital, Genova, Italy, 3University of Genoa, Genova, Italy, 4University of Geneva, Geneva, Switzerland, 5University of Genova, Genova, Canada**

**Background:** Severe COVID-19 outcomes have been reported in people living with HIV (PLWH). High SARS-CoV-2 RNAemia has emerged as a hallmark of severe COVID-19, yet its pathogenic role in the context of COVID-19 in PLWH is currently unknown. We hereby measured SARS-CoV-2 RNAemia and explored its association with T-cell/humoral responses and clinical severity in PLWH.

**Methods:** Unvaccinated PLWH and age/sex-matched people living without HIV (PLWHO) hospitalized for radiologically-confirmed COVID-19 pneumonia were consecutively enrolled (March 2020–January 2021). We measured: SARS-CoV-2 RNAemia (RT-qPCR); T-cell activation (HLA-DR+CD38+), cytotoxic T-cells [granzyme-B (GRZB)+perforin (PRF)+], GRZB/PRF production (MFI) by cytotoxic T-cells (flow cytometry); SARS-CoV-2-specific cytokines (IFN-γ, TNF-α, IL-2/IFN-γ, IL-10) producing T-cells, after SARS-CoV-2 spike peptide challenge (flow cytometry); anti-RBD antibodies (ELISA), Spike-ACE2 binding inhibition (receptor binding inhibition assay). Statistics: Mann-Whitney test and Spearman’s correlation.

**Results:** 18 PLWH (16 on cART; median CD4 636.5/mL; HIV-RNA< 50 cp/mL in 15/18) and 18 PLWHO were included at a median of 10 days from symptoms onset (Fig.1A). PLWH had lower P O2/ FIO2 (140 (122–151.5) vs. 207 (182–210)) and higher SARS-CoV-2 RNAemia (P<0.0007). PLWH showed lower T-cell activation (HLA-DR+CD38+) and cytotoxic T-cells [granzyme-B (GRZB)+perforin (PRF)+] (P<0.01) and lower GRZB/PRF production (MFI) (P<0.0007) compared to PLWHO. Anti-RBD antibodies (ELISA) were not detected, and Spike-ACE2 binding inhibition (receptor binding inhibition assay) was not statistically significant.
Figure 1

**345 HIGH-RESOLUTION MAPPING OF T-CELL HYBRID IMMUNITY TO THE ENTIRE SARS-CoV-2 PROTEOME**

Raúl Pérez-Caballero1, Laia Bernad2, Athina Kilpeläinen3, Oscar Blanch-Lombar1, Luis Romero1, Ruth Peña1, Gabriel Felipe Rodríguez-Lucano1, Josep Maria Manresa-Dominguez2, Bonaventura Clotet1, Alex Olvera1, Christian Trias i Pujol2

Fig.1A

To understand T-cell responses to SARS-CoV-2, it is essential to define the contribution of infection versus immunization to virus-specific hybrid immunity. Here, we characterized the breadth and magnitude of T-cell responses to the entire SARS-CoV-2 proteome over a 2-year follow-up period in infected and vaccinated (CoV2+Vac+) and vaccinated and infected (Vac+CoV2+) individuals.

**Methods:** We selected samples from 38 (19 CoV2+ and 19 CoV2-, time 1, T1) ProHePIc-19 cohort participants, a prospective, longitudinal study starting in March 2020 involving 7,776 healthcare workers in Spain. Longitudinal samples were available from 10 of them after a 3-dose mRNA vaccination, including 5 CoV2+Vac+ and 5 Vac+CoV2+, at 82.4 and 250.5 days from symptoms onset (DfSO, time 2, T2). We measured the breadth and magnitude of IFN-γ T-cell responses by ELSpot assay in cryopreserved PBMCs, using a 15-mer overlapping peptide (OLP) library of 2,790 SARS-CoV-2 peptides in 100 pools.

**Results:** We identified immunodominant T-cell responses in S1, S2, nsp3, Env, NC, and M proteins across the SARS-CoV-2 proteome. We observed an increased breadth of T-cell responses (responding pools over the entire region) to S1 (44–30%) and S2 (31–40%) in CoV2+Vac+ and Vac+CoV2+, respectively. In addition, CoV2+Vac+ had an exclusive and sustained response to M. We found significantly stronger responses in CoV2+Vac+ (P = 0.0313). Particularly, the total magnitude was greater in CoV2+Vac+ vs. Vac+CoV2+ in S1 (44.76.88 vs. 14.98, S3), Env (457.34 vs. 250.50), and M (455.13 vs. 0.00) but not in S2 and nsp3.

**Conclusion:** As compared to HIV-uninfected patients, PLWH hospitalized for COVID-19 pneumonia feature high SARS-CoV-2 RNAemia which is linked to respiratory failure and skewed T-cell responses, with higher perforin production by cytotoxic T-cells and SARS-CoV-2–specific T-cells, yet positively with perforin production by cytotoxic T-cells. No correlations between RNAemia and humoral responses were found.

**Figure 1**
**Methods:** Serum samples were tested using a multiplex bead-based immunoassay to measure antibody binding against 22 antigens including Nucleocapsid (N) and Spike (S) proteins of the 7 human coronaviruses and one malaria antigen. 

**Results:** We tested 819 longitudinal samples from 80 participants collected between July 2013 and May 2021 (3-16 samples per participant). Using a signal to noise ratio (S/N) > 0.1, 13, 1, and 5 participants showed at least one time point with IgG responses to 5 of SARS-CoV-2 (ancestral); SARS-CoV-1 and MERS-CoV respectively while 14, 8, and 11 participants showed responses to N before 2020. Across individuals, IgG binding to SARS-CoV-2 subunit S2 was most frequently detected and it showed the highest within-host fluctuations over time. A few individuals had elevated responses that persisted over years towards multiple antigens, most frequently to different SARS-CoV-2 antigens and rarely to distinct viruses. One individual showed high RBD-specific IgG response to distinct coronaviruses at a single time point before 2020. Responses against coronaviruses measured post-2020 generally correlated with responses measured before 2020, except for a subset of infected individuals whose responses against SARS-CoV-2 dramatically increased post-pandemic. IgG responses against the ancestral SARS-CoV-2 variant were most correlated with responses against Alpha and Gamma (then to Beta and Delta, rho > 0.75) variants. Using an IgG S/N > 10, 31 participants were Malaria positive and 22 showed concurrent elevated coronavirus IgG responses. However, about half of the malaria positive participants had no IgG responses against any coronavirus antigen and the rest presented limited and variable patterns of association between responses against coronaviruses and malaria.

**Conclusion:** Our study confirmed that a small subset of individuals in Africa had long-lasting IgG coronavirus-specific antibodies before the pandemic. While there was an association between coronavirus IgG responses and responses against malaria, there was no correlation between IgG responses and malaria infection. Further analysis is needed to better understand the interactions between antigens in the development of antibody immunity to coronaviruses.

**Table 1. Numbers of the coronavirus responders in IgG by country**

<table>
<thead>
<tr>
<th>Country</th>
<th>Total N</th>
<th>SARS-CoV-2</th>
<th>SARS-CoV-1</th>
<th>MERS-CoV</th>
<th>SARS-CoV-2</th>
<th>SARS-CoV-1</th>
<th>MERS-CoV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya</td>
<td>34</td>
<td>7 (21%)</td>
<td>3 (9%)</td>
<td>1 (3%)</td>
<td>3 (9%)</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Nigeria</td>
<td>5</td>
<td>5 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tanzania</td>
<td>9</td>
<td>9 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uganda</td>
<td>31</td>
<td>2 (6%)</td>
<td>2 (6%)</td>
<td>2 (6%)</td>
<td>2 (6%)</td>
<td>2 (6%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*The values in parentheses are the number of participants with S/N > 10 in more than half of the time points.*

348 IMPACT OF COVID-19 AND HOST FACTORS ON THE HUMORAL IMMUNE REPERTOIRE IN TREATED HIV

Samuel R. Schnittman, Wonyeong Jung, Kathleen V. Fitch, Markella V. Zanni, Sara McCallum, Jessica Lee, Sally Shin, Brandon J. Davis, Evelyne S. Fulda, Marissa Diggis, Judith S. Currier, Pam S. Douglas, Heather J. Ribaudo, Galit Alter, Steven K. Grinspoon

**Background:** People with HIV (PWH) on antiretroviral therapy (ART) appear to be at higher risk for worse COVID-19 outcomes, but the underlying mechanisms—including effects of COVID-19 and host factors on the broader humoral immune repertoire—are poorly understood.

**Methods:** REPRIEVE enrolled a global cohort of ART-treated PWH ages 40-75. COVID+ was defined by positive receptor binding domain IgG or IgA from annual visits in 2020-2021. Antibody isotype, subclass, and Fc receptor Luminex arrays to SARS-CoV-2, CMV, EBV, HSV, HIV, influenza, pneumococcus, and RSV were assessed. Report of COVID diagnosis (collected every 4 months) was defined to SARS-CoV-2, CMV, EBV, HSV, HIV, influenza, pneumococcus, and RSV visits 5/2020-2/2021. Antibody isotype, subclass, and Fc receptor Luminex arrays were collected prior to SARS-CoV-2 positivity (baseline) and then weekly for four weeks post-SARS-CoV-2 positivity.

**Results:** Of a total of 4,525 PWH who tested positive for SARS-CoV-2, 102 PWH had available data at all time points, and we compared changes in counts and percentages at each week post-SARS-CoV-2 to baseline values, using Wilcoxon rank sum test.

**Conclusion:** People with HIV (PWH) on antiretroviral therapy (ART) appear to be at higher risk for worse COVID-19 outcomes, but the underlying mechanisms—including effects of COVID-19 and host factors on the broader humoral immune repertoire—are poorly understood.

349 LYMPHOID TRAJECTORIES AND OUTCOMES OF COVID-19 INFECTION IN PEOPLE WITH HIV

Jerry Liu, Richard Medford, Roger Bedimo, John Hanna

**Background:** Studies have shown that lymphopenia and a decreased CD4/CD8 ratio are correlated with the severity of COVID-19 infections. As people with HIV (PWH) can have altered CD4/CD8 ratios at baseline, this study examined the relationship between lymphocyte and T-cell subsets with COVID-19 disease outcomes among PWH.

**Methods:** This retrospective study included adult PWH (identified by HIV ICD codes, HIV RNA or antibody results, or antiretroviral therapy use excluding pre-exposure prophylaxis) in the Optum COVID-19 EHR database with positive SARS-CoV-2 PCR or antigen tests from February 2020 to December 2021. Outcomes included 30-day hospitalization, ICU stay, mechanical ventilation, and death from COVID-19. Absolute lymphocyte counts and percent and CD4:CD8 ratios were collected prior to SARS-CoV-2 positivity (baseline) and then weekly for four weeks post-SARS-CoV-2 positivity. We examined lymphocyte trajectories in PWH who had available data at all time points, and we compared changes in counts and percentages at each week post-SARS-CoV-2 to baseline values, using Wilcoxon rank sum test.

**Results:** A total of 4,525 PWH who tested positive for SARS-CoV-2, 102 PWH had available lymphocyte counts at all study time points. Compared to non-hospitalized PWH (n=38), hospitalized PWH (n=64) and PWH who were in the ICU (n=32) or ventilator dependent (n=27) experienced a larger drop in lymphocyte percentage in the first two weeks post-SARS-CoV-2 diagnosis with only a partial recovery in subsequent weeks. In patients who died (n=19), lymphocyte percentage recovered even more slowly. Hospitalized PWH, as compared to non-hospitalized PWH, had a significant decrease in lymphocyte percentage post-SARS-CoV-2 infection in the first week (< 0.19 vs < 0.05; P < 0.001), second week (< 0.23 vs < 0.02; P < 0.001), third week (< 0.20 vs 0.00; < 0.001),
and fourth week (-0.10 vs 0.00; 0.001), a trend seen in the ICU, mechanically ventilated, and deceased groups as well (Table 1). By the first week, CD4/CD8 ratio in COVID-19 positive patients was lower in the deceased (-0.18 vs 0.00; p=0.4), ventilator dependent (-0.15 vs 0.00; p=0.2), and ICU (-0.15 vs 0.00; p=0.4) groups.

**Conclusion:** Our study showed that not only is lymphopenia a marker of COVID-19 disease severity in PWH but also a failure of lymphocyte percentage recovery is associated with worse outcomes. There was also a trend towards worse outcomes associated with a lower CD4/CD8 ratio in the first week after COVID-19 infection.

**Median Lymphocyte Percent Delta Change Post COVID-19 Test Positivity Among PWH**

<table>
<thead>
<tr>
<th>Lymphocyte Percent</th>
<th>Non-Hospitalized</th>
<th>Hospitalized</th>
<th>ICU</th>
<th>Non-ICU</th>
<th>Ventilator Support</th>
<th>Survived</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Week vs Pre-COVID</td>
<td>-0.05</td>
<td>-0.19</td>
<td>-0.11</td>
<td>-0.23</td>
<td>6.11</td>
<td>-0.34</td>
<td>-0.11</td>
</tr>
<tr>
<td>P-value*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second Week vs Pre-COVID</td>
<td>-0.12</td>
<td>-0.23</td>
<td>-0.07</td>
<td>-0.37</td>
<td>6.06</td>
<td>-0.45</td>
<td>-0.06</td>
</tr>
<tr>
<td>P-value*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third Week vs Pre-COVID</td>
<td>0.00</td>
<td>-0.20</td>
<td>-0.04</td>
<td>-0.32</td>
<td>6.03</td>
<td>-0.51</td>
<td>-0.02</td>
</tr>
<tr>
<td>P-value*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fourth Week vs Pre-COVID</td>
<td>0.00</td>
<td>-0.10</td>
<td>0.02</td>
<td>-0.32</td>
<td>6.01</td>
<td>-0.25</td>
<td>-0.01</td>
</tr>
<tr>
<td>P-value*</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 1. Median Lymphocyte Percent Delta Change Post COVID-19 Test Positivity Among PWH**

*Wilcoxon rank sum test*

**350 CHRONIC HIV INFECTION IMPACTS THE IMMUNE RESPONSE DURING ACUTE SARS-CoV-2**

Skye Opsteen, Tim Fram, Nathan Erdmann, Dustin M. Long

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**Background:** SARS-CoV-2 infection typically causes self-limited disease, but a subset of individuals experience more severe disease associated with respiratory manifestations, hospitalization and mortality. People living with HIV (PLWH) have shown to have chronic immune activation and inflammation despite effective antiretroviral therapy. During the COVID pandemic, PLWH were found to have an increased risk of hospitalization and mortality with acute COVID-19. The immune response driving these worsened outcomes in PLWH is not defined. We analyzed immune activation and exhaustion markers, as well as antigen specific T cell responses during acute COVID-19 in PLWH versus HIV-seronegative controls to determine the impact of chronic HIV infection and inflammation on acute COVID-19.

**Methods:** We performed flow cytometric analyses on peripheral blood mononuclear cells from: 1) PLWH with acute COVID-19 (HIV+COVID), 2) HIV-seronegative individuals with acute COVID-19 (COVID) and 3) pre-COVID-19 pandemic PLWH (HIV). COVID+ samples were collected at an average of 4.7 (range 0.16) and 5.5 (range 0-20) days post-symptom onset for the COVID and HIV+COVID cohorts, respectively. Cells were stained for surface markers of activation/exhaustion and intracellular cytokines (with and without SARS-CoV-2-specific antigen stimulation). Observed immune responses were correlated with disease severity.

**Results:** PLWH with acute COVID-19 had increased classical (CD14+) monocytes compared to HIV-seronegative individuals with acute COVID-19. The HIV+COVID cohort also had higher expression of activation (CD80, CD137) and exhaustion (PD1, TIGIT) markers on CD4+ and CD8+ T cells compared to HIV-seronegative individuals. SARS-CoV-2 antigen stimulation resulted in similar response (PD1, TIGIT) markers on CD4+ and CD8+ T cells compared to HIV-seronegative controls to determine the impact of chronic HIV infection and inflammation on acute COVID-19. The immune response driving these worsened outcomes in PLWH is not defined. We analyzed immune activation and exhaustion markers, as well as antigen specific T cell responses during acute COVID-19 in PLWH versus HIV-seronegative controls to determine the impact of chronic HIV infection and inflammation on acute COVID-19.

**Prior SARS-CoV-2 Infection Protects Against Symptomatic Common Cold Coronavirus (ccCoV)**

Jawen David, Janet Monero, Janice Weinberg, Manish Sagar

Boston University, Boston, MA, USA

**Background:** Current COVID-19 vaccines provide substantial protection against severe COVID-19, but they do not completely eliminate subsequent SARS-CoV-2 infections. We examined incidence of and immune differences against related but different common cold coronaviruses (ccCoVs) as a proxy for response against a future emerging CoV among those with SARS-CoV-2 infection, COVID-19 vaccination, or neither exposure.

**Methods:** We assessed incidence of ccCoV (229E, HKU1, NL63, OC43) and rhinovirus/enterovirus infections among those with documented prior SARS-CoV-2 infection (n=493), prior COVID-19 vaccine, but no SARS-CoV-2 infection (n=1,568), or no prior SARS-CoV-2 infection or vaccination (n=2,874). We conducted a retrospective review of all individuals at Boston Medical Center that underwent a comprehensive respiratory panel polymerase chain reaction (CRP-PCR) test from November 30, 2020 to October 8, 2021 to estimate infection incidence. A subset within each group was assessed for coronavirus specific humoral and cellular immune responses, via pseudovirus neutralization and peptide stimulation T cell assays. Comparisons among the three groups were done using Chi-square and multi-variate Cox-proportional hazards models.

**Results:** Incidence of symptomatic ccCoV was lower in those individuals with documented prior SARS-CoV-2 infection (1.0%) compared to those with COVID-19 vaccination (2.9%) or no prior SARS-CoV-2 exposure (1.8%, p = 0.01). Rhinovirus/enterovirus incidence was similar in all three groups (range 6.2 – 8.7%). Individuals with prior SARS-CoV-2 infection and those with previous COVID-19 vaccination had similar plasma neutralization against SARS-CoV-2, OC43, and 229E spike bearing pseudoviruses. SARS-CoV-2 (p = 0.01) and OC43 nucleocapsid (p = 0.02), but not spike specific peptides, yielded higher T cell responses in individuals with a prior SARS-CoV-2 infection as compared to those with COVID-19 vaccination or no prior SARS-CoV-2 exposure.

**Conclusion:** Prior SARS-CoV-2 infection, but not COVID-19 vaccination, protects against subsequent related but different ccCoV symptomatic infection. This protection against symptomatic ccCoVs may be mediated by cellular responses to non-spike proteins. Future pan-coronavirus vaccines could be improved by including both spike and non-spike antigens.

**352 RESPIRATORY DISEASE FACTORS LINK WITH REDUCED SARS-CoV-2 SUSCEPTIBILITY IN PWH**

Irene A. Abela1, Anthony Hauser2, Chloe Pasin1, Magdalena Schwarzmüller1, Huldrych F. Günthard1, Alexandra Trkola1, Roger Kouyos1

1University of Zurich, Zurich, Switzerland, 2University Hospital Zurich, Zurich, Switzerland

**Background:** Despite favorable vaccine responses of people with HIV (PWH), susceptibility to SARS-CoV-2 (SARS-CoV-2) infection and increased risk of COVID-19 in immunocompromised PWH continue to be of concern. Here, we searched the Swiss HIV Cohort Study (SHCS) with >9500 actively enrolled, optimally treated PWH to identify factors associated with SARS-CoV-2 infection in the pre-and post-vaccination era.

**Methods:** We utilized information on SARS-CoV-2 events reported to the SHCS in 2020-2021. To detect symptomatic infection, we screened pre-pandemic (2019) and
pandemic (2020-2021) bio-banked plasma for SCoV2 antibodies (Ab). SCoV2+ and matched SCoV2- PWH were additionally screened for Abs to circulating human coronaviruses (HCoV). Data were compared to HIV negative (HIV-) controls. SCoV2 data and >26 behavioral, immunologic and disease-parameters available in the SHCS data base were analyzed by logistic regression, conditional logistic regression, and Bayesian multivariate regression.

Results: Considering information on the SCoV2 status of 6270 SHCS participants, neither HIV-1 viral load nor CD4+ T cell levels were linked with increased SCoV2 infection risk. COVID-19 linked SCoV2 case fatality rates (8/982) were low, but slightly higher than in the general Swiss population when stratified by age. Compared to HIV-, PWH had lower SCoV2 IgG responses (median effect size=−0.48, 95%-Credibility-Interval=[−0.7, −0.28]). Consistent with earlier findings, high HCoV Abs pre-pandemic (2019) were associated with a lower risk of a subsequent SCoV2 infection and, in case of infection, with higher Ab responses. Examining behavioral factors unrelated to the HIV-status, people living in single-person households were less at risk of SCoV2 infection (aOR= 0.77 [0.66,0.9]). We found a striking, highly significant protective effect of smoking on SCoV2 infection risk (aOR= 0.46 [0.36,0.56], p=2.6*10^-14) which was strongest in 2020 prior to vaccination and was even comparable to the effect of early vaccination in 2021. This impact of smoking was highly robust, occurred even in previous smokers and was highest for heavy smokers.

Conclusion: Our unbiased cohort screen identified two controversially discussed factors, smoking and cross-protective HCoV responses to be linked with reduced susceptibility to SCoV2, validating their effect for the general population. Overall weaker SCoV2 Ab responses in PWH are of concern and need to be monitored to ensure infection- and vaccine-mediated protection from severe disease.

353 DELINEATING FACTORS ASSOCIATED WITH ASYMPTOMATIC PRIMARY SARS-CoV-2 INFECTION

Irene A. Abela1, Karen Zafilaza2, Eve Todesco3, Cathia Souillé1, Antoine Fauchois1, Quentin Le Hingrat1, Anne-Geneviève Marcelin1, Vincent Calvez2, Charlotte Charpentier1, Diane Descamps1, Stephane Marot1, Valentin M. Ferre1
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Background: SARS-CoV-2 infection leads to a broad range of clinical manifestations, from no symptoms to critical illness. Post-stimulated innate defenses, rapid induction of SARS-CoV-2 responses and pre-existing cross-reactive immunity to circulating human coronaviruses (HCoV) may early dampen SARS-CoV-2 infection, preventing symptomatic disease. Here we explore SARS-CoV-2 and HCoV antibody impact on asymptomatic infection in individuals first encountering SARS-CoV-2 in March 2020-March 2021 participating in a longitudinal pediatric cohort (n=882) and a cross-sectional adult and children diagnostic cohort (n=882) in Switzerland.

Methods: Antibodies (Ab) to S1 of the four HCoV strains and SARS-CoV-2 antigens S1, RBD, S2 and N were determined in saliva (n=4993) and serum (n=7486) samples. Mucosal and systemic neutralization activity against wildtype SARS-CoV-2-Wuhan, and Alpha, Delta and Omicron (BA.2) variants of concerns (VoC) was assessed by pseudovirus neutralization in a subset of individuals.

Results: Analysis of simultaneously sampled saliva and plasma revealed a strong correlation of mucosal and systemic SARS-CoV-2 anti-spike responses in recent infection. Notably, pre-existing high HCoV antibody response was significantly associated with higher systemic (IgG, IgA, and IgM, p<0.001) and mucosal (IgG, p=0.03) SARS-CoV-2 responses following SARS-CoV-2 infection. Adjusting for age and sex, we found four saliva SARS-CoV-2 Ab parameters, namely total IgG, total IgG S1, IgG S2 and IgM S1 (p<0.001, p=0.01, p<0.02, p=0.01 respectively), inversely associated with salivary viral load highlighting the impact of mucosal Ab response on viral suppression. Saliva neutralization activity was modest but surprisingly broad, retaining same level activity against Wuhan, Alpha and Delta, but not Omicron BA.2, whereas plasma neutralization activity showed the typical decrease for all three VoC compared to Wuhan. Most interestingly, asymptomatic individuals presented with higher IgG S2 Ab reactivity in saliva (p=0.03) and higher pre-existing HCoV-S1 IgG activity in plasma (HKU1, p=0.04) and saliva (total hCoV, p=0.02) suggesting immune factors that restrict disease severity.

Conclusion: Focusing on a SARS-CoV-2 infection and vaccine naïve population, our study reveals interdependencies of systemic and mucosal SARS-CoV-2 and HCoV immunity following primary SARS-CoV-2 infection and locates reactivities linked with reduced viral load and asymptomatic infection.

HIGHER SPIKE GENEVICH GENTRICITY DE GTA/OMICRON VARIANTS IN IMMUNOCOMPRIMED HOSTS

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Background: Immunocompromised hosts with prolonged SARS-CoV-2 infections have been associated with the emergence of novel mutations, especially in the Spike protein, a key target for vaccines and therapeutics. Here, we conducted a case-control study to measure the genetic diversity of SARS-CoV-2 and to search for immunocompromised-specific minority variants.

Methods: SARS-CoV-2-positive patients with lung/cardiac/kidney transplant, HIV-positive, or treated with high doses of corticosteroids for autoimmune diseases were considered as immunocompromised hosts. SARS-CoV-2-positive healthcare workers with no auto-immune disease were used as controls. Samples were analyzed by RT-qPCR at Pitié-Salpêtrière and Bichat Claude-Bernard university hospitals (Paris, France). Samples with cycle threshold ≤30 were selected for SARS-CoV-2 whole-genome sequencing using Oxford Nanopore protocol. Raw sequence data were mapped onto the Wuhan-Hu-1 reference genome, and consensus sequences were produced to determine the lineage. Only sequences covering at least 95% at ≥30X depth of the Spike gene were investigated. In-house algorithms were developed to identify all majority and minority mutations in Spike. We defined a minority variant when it was present in ≥6% and <50% of the reads; and a majority variant when it was present in >50%.

Results: We sequenced SARS-CoV-2 genome from 478 COVID-19-positive immunocompromised patients and 234 controls. More minority non-synonymous mutations in Spike were detected in viruses from immunocompromised hosts, compared to viral genomes from controls, in both Delta (p=0.001) and Omicron (p=0.001) lineages, but not in Alpha (p=0.66) (Figure 1). Interestingly, among the 52 patients infected with the Delta variant, we concomitantly detected at low frequencies the mutations H655Y, N674K, D796Y, in three patients (associated with different auto-immune disease), that are part of Omicron variants signature mutations. Similarly, some patients (n=7) infected by Omicron BA.1 lineage had R346T at low-frequency, later fixed in Omicron BA.4.6 and BQ.1.1 lineages. None of these mutations were observed in the viral genomes from controls.

Conclusion: Here, we report a higher genetic diversity in Spike gene among SARS-CoV-2 sequences from immunocompromised hosts for Delta and Omicron lineages. These results suggest that immunocompromised patients are more likely to allow viral genetic diversification and are associated with a risk of emergence of novel SARS-CoV-2 variants.

Variable levels of Spike gene evolution for different SARS-CoV-2 lineages in immunocompromised hosts and care workers "controls" without autoimmune disease.
Background: Limited Covid-19 vaccine effectiveness (VE) studies address the mRNA, adenosine vector-based, and protein subunit vaccines and their mix and match in real-world settings. BNT162v2 (Pfizer-BioNTech), mRNA-1273 (Moderna), AZD1222 (AstraZeneca), and locally produced MCV-COV1901 (Medigen) are provided under the National Vaccination Program. Taiwan maintained a low circulation of Covid-19 infection until a major epidemic in Omicron BA.2, began in April 2022. The study aimed to estimate the VE against moderate and severe (severity) and fatal diseases (death) associated with SARS-CoV-2 among individuals administered one, two, and three doses of vaccination and categorized by vaccine type combinations in this predominantly infection-naive population.

Methods: The study included CDC’s administrative records from National Immunization Information System and National Infectious Disease Reporting System from March 21, 2021, to September 30, 2022. Criteria for Covid-19 severity followed WHO’s guidelines, and the committee reviewed the records. The study calculated each individual’s last administered date to disease onset (incidence rate (IR) per 100,000 person-days) to explore the incidence rate to compare the presence of risk probabilities. Multiple logistic regression was used for vaccine effectiveness analysis.

Results: Of 23,933,482 individuals included in the study, and 6,202,496 infections, 30,976 severity, and 10,851 deaths were observed. Compared with three doses administered, three doses of AZD1222 or it as primary series plus mRNA or protein-based vaccines were at higher risk of severity (IR: 0.390-0.762), followed by mRNA-1273 (IR: 0.316-0.471), MCV-COV1901 (IR: 0.044-0.196) and BNT162v2 (IR: 0.061-0.197). As for the death outcome, AZD1222 was at higher risk (IR: 0.127-0.269), followed by mRNA-1273 (IR: 0.086-0.125), MCV-COV1901 (IR: 0.013-0.064) and BNT162v2 (IR: 0.015-0.045). VE against the severity of AZD1222 or it as primary series ranged from 65.9% to 77.7%; mRNA vaccines ranged from 86.4% to 96.1%; and protein-based vaccines ranged from 91.4% to 96.2%. A similar pattern of VE against death ranged from 70.9% to 77.3%, 78.2% to 84.2%, and 77.1% to 93.5% for mRNA, protein subunit, and inactivated virus vaccines, respectively.

Conclusion: Individuals who received their primary series as AZD1222 might not have adequate protection against Covid-19 severity so encourage those vulnerable groups to receive additional booster doses. The study also indicated that protein subunit vaccines provide similar protection against severity and death as mRNA vaccines.

Vaccine Effectiveness and Severity and Death Incidence Rate by Vaccine Type and Mix-and-Match Groups

- **Primary series:** AZD1222 (AstraZeneca)
  - AZ + AZ: 11,592 (63.9%) 0.366 (62.8%)
  - AZ + mRNA: 4,652 (11.4%) 0.259 (61.1%)
  - AZ + BNT: 3,155 (3.4%) 0.224 (70.3%)

- **Primary series:** Moderna (Moderna)
  - Moderna + mRNA: 2,812 (6.4%) 0.345 (87.4%)
  - Moderna + BNT: 2,106 (3.8%) 0.256 (74.1%)

- **Primary series:** BNT (BioNTech)
  - BNT + mRNA: 3,106 (6.4%) 0.312 (87.5%)
  - BNT + Moderna: 1,676 (3.2%) 0.388 (68.9%)

- **Primary series:** MCV-COV1901 (Medigen)
  - MCV + Moderna: 3,091 (6.4%) 0.345 (91.9%)
  - MCV + BNT: 2,719 (5.2%) 0.345 (91.9%)

Conclusion: mRNA and protein subunit vaccines were at higher risk of severity and death as compared to inactivated virus vaccines. AZD1222 vaccine, when used as the primary series, was associated with higher risk of severity and death compared to mRNA and protein subunit vaccines.

VSV-based COVID vaccine for the prevention of breakthrough SARS-CoV-2 Infections

**Methods:** We have used a recombinant vesicular stomatitis virus (rVSV)-based prime-boost immunization strategy to develop an effective COVID-19 vaccine candidate. We have constructed an attenuated recombinant VSV genome carrying the full-length Spike protein gene of SARS-CoV-2. Adding the hemagglutinin signal peptide (mSP) at the N-terminus enhanced the protein expression and adding the VSV G protein transmembrane domain and the cytoplasmic tail (Gtc) at the C-terminus of the Spike protein allowed efficient incorporation of the Spike protein into pseudovirus particles.

**Results:** In immunized mice, rVSV with chimeric rVSV-msp-S-Gtc induced high levels of potent neutralizing antibodies (nAbs) and CD8+ T-cell responses, while the full-length Spike with Gtc proved to be the superior immunogen. More importantly, rVSV-msp-S-Gtc vaccinated animals were completely protected from subsequent SARS-CoV-2 challenges. Furthermore, rVSV-Wuhan and rVSV-Delta vaccines, and an rVSV-Trivalent (mixed rVSV-Wuhan, -Beta and -Delta) vaccine elicited potent nAbs against live SARS-CoV-2 Wuhan (USAWA1), Delta (B.1.617.2) and Omicron (B.1.1.529) viruses. Heterologous boosting of rVSV-Wuhan with rVSV-Delta induced strong nAb responses against Delta and Omicron viruses, with the rVSV-Trivalent vaccine consistently inducing effective n Abs against all the SARS-CoV-2 variants tested. All rVSV-msp-S-Gtc vaccines also elicited an immunodominant Spike-specific CD8+ T-cell response.

Conclusion: rVSV vaccines targeting SARS-CoV-2 variants of concern can be considered as an effective booster vaccine in the global fight against COVID-19.
HOSPITAL PROTECTION CORRELATES IN OUTPATIENT COVID-19
CONVALESCENT PLASMA RECIPIENTS
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Background: High titer COVID-19 convalescent plasma (CCP) reduces hospitalizations among immunocompetent outpatients. This study evaluated recipient post-transfusion S receptor binding domain (S-RBD) IgG antibody levels and the association of progressing to hospitalization among unvaccinated outpatients with COVID-19 treated with CCP or control plasma.

Methods: This analysis focused on participants from a multicenter double-blind, randomized, controlled trial comparing treatment of outpatients with COVID-19 convalescent plasma (CCP) or control plasma without SARS-CoV-2 antibodies. Participants with confirmed SARS-CoV-2 infection were transfused within 9 days of symptom onset between June 2020 and October 2021 (n=110 vaccinated control; n=105 vaccinated CCP; n=464 unvaccinated control; n=472 unvaccinated CCP; total n=574 control and n=577 CCP recipients). All subjects had specimens collected the day prior to transfusion (D-1), within 30 minutes after transfusion (D0), 14 (D14), 28 (D28), and 90 (D90) days post-transfusion. Ancestral SARS-CoV-2 S-RBD was measured by an in-house validated ELISA. All S4 COVID-19-related hospitalizations occurred within 2 weeks of transfusion.

Results: Post-transfusion anti-S-RBD IgG levels on D0 were significantly greater for CCP (median=4 titer,log3) compared to control (median=2 titer,log3; p < 0.001) recipients. Neither sex nor age impacted antibody levels following CCP treatment at D14, D28, and D90. Vaccinated recipients had greater titers than unvaccinated recipients prior to transfusion with little change in titers post-transfusion. Unvaccinated recipients had low antibody titers on D-1 with CCP recipients exhibiting a significant increase in titer from D-1 to D0 compared to controls (mean fold change=1.89; p < 0.001). Among unvaccinated recipients, those who received CCP transfusion late (>5 days after symptom onset) and had low D0 antibody levels (< 4.24 titer, log3) had the greatest proportion of hospitalizations (5.5%). In contrast, those who received CCP transfusion early (< 5 days after symptom onset) and had high D0 antibody levels (> 4.24 titer, log3) had no hospitalizations. Unvaccinated CCP recipient anti-S-RBD IgG antibody levels on D0 correlated with donor anti-S-RBD IgG antibody levels (r=0.30, p < 0.001).

Conclusion: Among unvaccinated outpatients with COVID-19, CCP recipient antibody dilutional titers after transfusion over 540 titer correlated with protection against hospitalization when transfusion occurred within 5 days of symptom onset.

Figure of the proportion of hospitalizations by early vs. late transfusion and high vs. low recipient antibody levels.
Results: SARS-CoV-2 mRNA vaccination provoked strong T cell clonal expansion in most participants. At 8-12 months after infection, each primary mRNA dose increased the abundance and diversity of S-specific T cells. Clonal and integrated expansions were larger in CD8+ than in CD4+ T cells. At the convalescent time point, we observed greater diagnostic S-reactive CD4 T cell breadth in hospitalized vs. non-hospitalized patients (p<0.01). CD4 T cell breadth was again higher in previously hospitalized persons after the 2nd primary (p=0.02) and booster (p<0.01) doses, suggesting that diverse CD4 T cell memory after severe infection leads to increased repertoire diversity after vaccination. S-specific T cells with identical TCRs were detectable in blood and the nasal mucosa, with specificity confirmed using a TRA/TRB transgenic T cell with the matching receptor.

Conclusion: Although both S-specific CD8 and CD4 T cell memory are established by prior infection, S-specific CD8 T cells predominated in blood after primary vaccination, with some clonotypes showing up to 1000-fold expansion across 1-2 mRNA doses. Vaccine-reactive CD8 clonotypes were present at the barrier nasal site after booster mRNA dosing. Severe disease impaired a highly diverse S-reactive CD4 repertoire persisting through vaccination.

361 LIMITED INDUCTION OF LUNG-RESIDENT MEMORY T CELLS BY SARS-CoV-2 mRNA VACCINATION
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Background: Resident memory T cells (Tcm) present at the respiratory tract may be essential to enhance early SARS-CoV-2 viral clearance, thus limiting viral infection and disease. While long-term antigen-specific Tcm are detectable beyond 11 months in the lung of convalescent COVID-19 patients after mild and severe infection, it is unknown if mRNA vaccination encoding for the SARS-CoV-2 S-protein can induce this front-line protection.

Methods: We obtained cross-sectional paired blood and lung biopsy samples from patients (n=30) undergoing lung resection for various reasons and assigned them to one of four groups: I) uninfected unvaccinated individuals (n=5), II) unvaccinated long-term SARS-CoV-2 convalescent individuals (between 6.0-10.5 months post-infection; n=9), III) uninfected and long-term vaccinated individuals (between 6.0-7.7 months after the second or third dose; n=10), and IV) uninfected and short-term vaccinated individuals (between 1.3-1.8 months after the third or fourth dose; n=6). We determined the presence of SARS-CoV-2-specific CD4+ and CD8+ T cells in blood and lung samples after exposure of cells to M, N, and S peptide pools, followed by flow cytometry to detect Tcm cells expressing interferon (IFN)γ and/or CD107a, as a degranulation marker.

Results: We found that the frequency of CD4+ T cells secreting IFNγ in response to S-peptides was variable but detectable in blood and lung up to 8 months after mRNA vaccination. Moreover, the IFNγ response of CD4+ T cells in the lung to S-peptides was variable but detectable in blood and lung up to 8 months after mRNA vaccination. The CD40.SARS-CoV2 vaccine specifically elicits huCD8 Tscm cells.

Conclusion: mRNA vaccines might induce memory responses within the lung parenchyma in some patients, potentially contributing to the overall disease control. However, the robust and broad Tcm response established in convalescent-infected individuals may offer advantages at limiting disease if the virus is not blocked by initial mechanisms of protection, such as neutralization. Our results warrant investigation of mucosal vaccine-induced resident T cell responses in establishing superior site-specific protective immunity.

362 CD40.SARS-CoV2 VACCINE, BUT NOT mRNA, INDUCES SPECIFIC CD8+ T MEMORY STEM CELLS
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Background: Vaccination plays a major role in controlling SARS-CoV-2 infection but faces the issue of short-term protection. Beyond the generation of Abs, induction of memory CD8+ T cells with stem cell-like (Tscm) properties is essential for long-term immunity to viruses. We have designed a sub-unit CD40-CoV2 vaccine which targets Spike (S) and nucleocapsid (N) regions from SARS-CoV-2 to antigen presenting cells with comparable immunogenicity and protective effect than mRNA BNT162b2 (Pfizer-BioNTech) in preclinical models (Colón S. EBioMed 2022). We hypothesized that CD40-CoV2 vaccine will elicit CD8+ T cells.

Methods: CD40-CoV2 vaccine is a fully humanized mAb fused to RBD (aa 318-541) and N (aa 276-411). Humanized (hu) NSG mice (HIS-mice) (n=6-10) were given: i) CD40-CoV2 (10 μg equal to 1.3 μg of RBD, i.p.) +/- poly-CLLC (TLR3 agonist; 50μg) or ii) BNT162b2 (1μg, i.m.); iii) IgG4-CoV2 (10μg, i.p.) as non-CD40-targeting control. Phenotype and function of splenic S- and N-specific T cells were assessed at W5.

Results: The CD40-CoV2 vaccine +/- poly-CLLC induced significant S and N-specific Th1 huCD4+ T cells and RBD-specific IgG-switched huB cells as compared to mock injections and non-targeted IgG4-CoV2. CD40-CoV2 vaccine +/- adjuvant induced higher frequencies of huCD8+ Tcm (CD95+ CD45RA+ CD62L+; median, (IQR) 22.4% (12.3-27.4) and 23% (20.7-29.1) +/- adjuvant, respectively) and central memory (Tcm) (CD45RA-CD62L+CD4+ T cells (2.7% (2.3-6.2) and 5.1% (3.8-7.8) +/- adjuvant, respectively). In contrast, BNT162b2 induced predominantly effector memory (Tem) (CD45RA-CD62L- median, (IQR) 63.1% (47.3-72.3) but not Tcm (1.6% (0.9-6.6)). CD40-CoV2 induced huCD8+ T cells exhibit i) a higher proliferation index than Tem and Tcm, ii) a functional profile secreting TNF and IFNγ after restimulation with RBD or N peptides; and iii) features of Tem cells.

Conclusion: The CD40.SARS-CoV2, but not BNT162b2 vaccine, stimulates selective enrichment in S- and N-specific CD8+ T cells that support long-lasting anti-viral immunity. CD40-CoV2 sub-unit is under clinical development as a booster vaccine aimed to maintain durable anti-viral T and humoral responses. The CD40 SARS-CoV2 vaccine specifically elicits huCD8 Tscm cells.
Results: All individuals had detectable levels of spike-specific CD4+ T cells at T2, while only 72.2% of individuals had detectable levels of spike-specific CD6+ T cells. Treatment type did not significantly impact the magnitude or phenotype of T cell responses, including those to Omicron. However, increased age was associated with decreased ancestral CD6+ T cell responses at T2.

Further, ancestral and omicron responses were significantly different at T2, with decreased magnitude and altered phenotype of omicron-specific CD4 + T cells.

Conclusion: Here, we report that solid tumor patients, treated with either chemotherapy or biologics, mount robust T cell immunity to SARS-CoV-2 following vaccination. Additional data is needed to determine if these responses correlate with antibody levels and clinical illness.

364 NEUTRALIZING ACTIVITY AND T CELL RESPONSE AFTER BIVALENT THIRD BOOSTER DOSE IN PLWH
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Background: Aim of the study was to analyze neutralizing activity against BA.5, BQ.1.1 and T cell response after 3rd booster dose [3BD (5th shot)] with BA.4/5 bivalent vaccine by hybrid immunity (HI) and CD4 count in advanced PLWH.

Methods: In PLWH with previous AIDS and/or CD4< 200/mm³ receiving 3BD (original strain/BA.4/5), immunogenicity was assessed at time of 3BD (T0) and at day 15 (T1) by microneutralization assay [MNA90] against Omicron BA.5, BQ.1.1 and by IFNγ ELISA. PLWH were stratified by HI vs. nHI and by CD4 count at T0 (>or< 500/mm³). For crude mean comparisons, neutralizing antibodies (nAbs) were expressed in natural scale and fold changes, IFNγ and all values for regression analyses in log, scale, paired t-test used to test changes over T0-T1. Two 2-arms parallel trials were emulated: HI and CD4 count as exposure, log 2 comorbidity, 87% with previous AIDS, median CD4 nadir 44 cell/mm³ (16-102), years from AIDS; when HI was the exposure also CD4 count). Regression analyses in log 2 scale, paired t-test used to test changes over T0-T1. Two 2-arms parallel trials were emulated: HI and CD4 count as exposure, log, nAbs and IFNγ as outcome. Average treatment effect (ATE) of the two exposures were estimated by marginal models weighted for potential confounders (age, CD4 nadir, years from AIDS; when HI was the exposure also CD4 count). No evidence was found that these responses correlate with antibody levels and clinical illness.

Conclusions: We observed strong neutralization against BA.5, and retained cross-neutralization against BQ.1.1 and BA.5, with higher neutralizing response against BA.5 [ATE=1.17 log2 (95%CI 0.34;2.00), P=0.006] but not against BQ.1.1 [ATE=1.17 log2 (95%CI 0.34;2.00), P=0.006].

365 12-MONTH HUMORAL RESPONSE AFTER mRNA COVID-19 VACCINATION IN PEOPLE LIVING WITH HIV
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Background: We compared the 12-month post primary vaccination humoral immune response to mRNA COVID-19 vaccines in PLWH and controls.

Methods: PLWH and HIV-negative healthy controls included in the French national multi-center prospective COVID 19 vaccine cohort study ANRS00015 COV-POPART were analyzed. Percentage (95% CI) of responders (positive anti-Spike SARS-CoV-2 IgG antibodies) and geometric means titers (95% CI) of anti-Spike SARS-CoV-2 IgG antibodies (BAU/mL) were assessed at 1 month (M1), 3 months (M3) and 6 months (M6) after the 2nd dose of the primary vaccination and at 12 months in those who received a booster dose. Specific neutralizing antibodies (nAbs) (in vitro neutralization assay against original, Delta and Omicron BA.1.1 strains) were estimated in a subset of participants. Serological tests (ELISA Euroimmun) and seroneutralization were performed centrally.

Results: Overall, 858 PLWH and 1156 controls were included. PLWH were older than controls: 55.2 years, (49.6-60.6) vs 46.6 years (36.5-56.6) and more frequently male (75.1% vs 48.9%). Among PLWH at inclusion, 97.3% were under antiretroviral therapy, 96.5% had an undetectable viral load and 71.8% had CD4 counts above 500 cells/mm³. Participants had namely received BNT162b2 as the primary vaccination (93% in PLWH vs 84% in controls) and 53.1% had received a booster dose (57.2% in PLWH (median time after the 2nd dose: 6.1 M [5.9-6.7]) and 50.1% in controls (median time 6.0 M [5.5-6.2]). Percentage of responders after the 2nd dose was lower in PLWH than controls (98.7% [97.7; 99.3] vs 99.9% [99.5; 99.9], p=0.0001). PLWH had significantly lower levels of anti-Spike antibodies at 1 M (1188 [650; 2067] vs 1506 [950; 2507] BAU/mL, p<0.0001) and 6 M (149 [95; 235] vs 194 [124; 314] BAU/mL, p<0.0001) but similar levels at 12 M (520 [269; 1190] vs 427 [239; 1087] BAU/mL, p=0.3367) (Figure A). PLWH had significantly lower nAbs against original, Delta and Omicron BA.1.1 strains at 1, 6 and 12 M after primary vaccination compared to controls. The booster dose significantly increased the titers of nAbs against original and Delta strains and, to a lower extent, against Omicron (Figure B).

Conclusion: PLWH had high response rates to mRNA COVID-19 vaccines but lower titers of antibodies and nAbs at 1 and 6 M after primary vaccination than controls. One mRNA booster dose increased SARS-CoV-2 IgG antibodies titers...
to similar levels to controls but neutralizing activity especially against Omicron remained lower. Median anti-Spike antibodies (A) and seroneutralization (B) titers at 1, 6 and 12 months following mRNA Covid-19 primary vaccination in PLHIV and controls.

366 SARS-CoV-2 LIVE VIRUS NEUTRALIZATION AFTER FOUR COVID-19 VACCINE DOES IN PWH ON ART

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Background: Limited data exist regarding the immune benefits of fourth COVID-19 vaccine doses in people with HIV (PWH) receiving antiretroviral therapy (ART), particularly given that most have now experienced SARS-CoV-2 infection. We measured the effect of fourth doses on SARS-CoV-2 neutralization in 63 PWH, including 19 SARS-CoV-2-naive and 44 SARS-CoV-2-experienced participants.

Methods: Wild-type (WT), Omicron-BA.5 and Omicron-BQ.1-specific neutralization activities were longitudinally quantified using live virus assays up to one month post-fourth vaccine dose. Multiple linear regression was used to investigate the relationship between sociodemographic, health and vaccine-related variables and SARS-CoV-2 neutralization.

Results: Participants (54 male; 9 female) received monovalent (44%) or bivalent (56%) mRNA fourth doses. In COVID-19-naive PWH, a fourth dose enhanced WT- and BA.5-specific neutralization modestly above three-dose levels (p=0.1). In COVID-19-experienced PWH, a fourth dose enhanced WT neutralization modestly (p=0.1) and BA.5 neutralization significantly (p=0.002). Consistent with the humoral benefits of ‘hybrid’ immunity, the highest neutralization was observed in COVID-19-experienced PWH after a fourth dose. Of note, PWH with Omicron-era infections exhibited higher WT-specific (p=0.04), but comparable BA.5- or BQ.1-specific neutralization, compared to PWH with pre-Omicron-era infections. Overall, BA.5 neutralization was significantly lower than WT in all participants regardless of COVID-19 experience, and BQ.1 neutralization was significantly lower than BA.5 (all p<0.0001). In multivariable analyses, fourth dose valency did not significantly affect neutralization magnitude, nor did sex, age nor CD4+ T-cell count (neither recent nor nadir). Rather, an mRNA-1273 fourth dose (versus a BNT162b2 one) was the strongest correlate of WT-specific neutralization, while prior COVID-19, regardless of infection era, was the strongest correlate of BA.5 and BQ.1-specific neutralization post-fourth dose.

Conclusion: Fourth COVID-19 vaccine doses, irrespective of valency, benefit PWH regardless of prior SARS-CoV-2 infection, but the highest neutralization of Omicron-BA.5 and BQ.1 variants post-fourth dose occurred in PWH with hybrid immunity. These results support existing recommendations that all adults receive a fourth immunization within 6 months of their third vaccine dose (or their most recent SARS-CoV-2 infection).

367 IMMUNOGENICITY OF SARS-CoV-2 mRNA VACCINATION IN IDIOPATHIC CD4 LYMPHOPENIA

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Background: The rapid development of SARS-CoV-2 mRNA vaccines has been a remarkable success of the COVID-19 pandemic, but vaccine-induced immunity is heterogeneous in immunocompromised populations. We sought to determine the immunogenicity of SARS-CoV-2 mRNA vaccines in a cohort of people with idiopathic CD4 lymphopenia (ICL).

Methods: 25 patients with ICL followed at the National Institutes of Health on a natural history protocol were evaluated between 2020-2022. Blood and serum was collected within 4-12 weeks after their second and/or third SARS-CoV-2 mRNA vaccine dose. Twenty-three matched healthy volunteers (HVs) provided blood samples at similar timepoints post-mRNA vaccination on a separate clinical protocol. Pre-vaccine blood samples were also used when available. Anti-spike and anti-receptor binding domain antibodies were measured. T-cell stimulation assays were performed to quantify SARS-CoV-2 specific T-cell responses. Comparisons were made with Wilcoxon test.

Results: Twenty-three participants with ICL had samples collected after their second mRNA vaccine and 7-individuals after the third dose. Median age at vaccination was 51-years (IQR: 44-62) and 12 were women (48%). Median CD4 T-cell count was 150 cells/μL (IQR: 85-188) at the time of vaccination, and 11-individuals (44%) had a baseline CD4 count ≤100 cells/μL. HVs had a median age of 54-years (IQR: 43-60) with 13-women (56%). Anti-spike IgG antibodies were significantly higher in HVs than with those ICL after 2-doses. Lower SARS-CoV-2 IgG antibody production was primarily observed in those with baseline CD4 T-cells ≤100 cells/μL (Figure-1A). The decreased production in ICL remained after a third vaccine dose (Figure-1B). There was a significant correlation between anti-spike IgG and baseline CD4 count. Spike-specific CD4 T-cell responses in volunteers compared to those with ICL demonstrated similar levels of activation induced markers (CD154+CD69+) and cytokine production (IFNγ+, TNFα+, IL-2+) after two or three mRNA vaccine doses. Quantitatively the smallest responses were observed in those with lower baseline CD4 T-cells (Figure 1C-D). Minimal SARS-CoV-2 CD8 T-cell responses were detected in both groups.

Conclusion: Patients with ICL and baseline CD4 T-cells ≤100 mount similar humoral and cellular immune responses to SARS-CoV-2 vaccination as healthy volunteers. Those with baseline CD4 T-cells ≤100 have impaired vaccine-
induced immunity and should be prioritized to additional boosters and continue other risk mitigation strategies.

Figure 1: SARS-CoV-2 specific antibody and T-cell responses after mRNA vaccination.

### 368 RESTORATION OF HUMORAL RESPONSE TO COVID-19 VACCINATION IN RITUXIMAB-TREATED PATIENTS

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**Background:** Previous studies have demonstrated that patients with hematologic malignancies had suboptimal antibody response after receiving COVID-19 vaccines, especially among those having previously treated with anti-CD20 monoclonal antibodies.

**Methods:** Adult patients with non-Hodgkin's lymphoma or chronic lymphocytic leukemia (CLL) were enrolled before receiving the second dose of SARS-CoV-2 vaccine. Determinations of anti-SARS-CoV-2 spike and nucleocapsid IgG titers were performed every 1–3 months after they received the second and the third dose of SARS-CoV-2 vaccine, respectively. Patients were excluded from analysis if they were diagnosed with COVID-19. All serum samples were tested for anti-nucleocapsid antibody and those tested positive were excluded from subsequent analyses.

**Results:** A total of 85 participants were enrolled, including 42 (49.4%) with diffused large B-cell lymphoma, and 13 (15.3) with follicular lymphoma and 9 with CLL. 72 (84.7%) participants had received anti-CD20 monoclonal antibodies, with a median interval of 24 months between last anti-CD20 treatment and the second dose of vaccine, and 21 (24.7%) had HIV infection. Factors associated with failure to achieve an anti-spike IgG titer >141 BAU/mL within 12 weeks after the second dose of vaccine included HIV infection (adjusted odds ratio [aOR], 0.14; 95% CI, 0.04–0.51), active hematologic disease (aOR, 5.50; 95% CI 1.42–21.32), receipt of anti-CD20 monoclonal antibodies (aOR, 6.65; 95% CI 1.52–29.07), and receipt of two doses of homologous mRNA vaccination (aOR, 0.17; 95% CI 0.05–0.56).

In the participants having previously treated with anti-CD20 regimen, only 8.6% achieved an antibody response (>141 BAU/mL) in the first year, while 78.3% achieved anti-spike IgG titer > 141 BAU/mL after two years post-CD20 depletion treatment. After the third dose of SARS-CoV-2 vaccine, 53.6% achieved an anti-spike IgG titer > 141 BAU/mL in the first year post-CD20 treatment.

**Conclusion:** Our study demonstrated that previous treatment with anti-CD20 monoclonal antibodies was associated with a lower antibody response among patients with lymphoproliferative disorders receiving two doses of SARS-CoV-2 vaccine. While two doses of SARS-CoV-2 vaccines might not be sufficient even one year apart from the last dose of rituximab, a third dose of vaccine may boost anti-spike IgG particularly in the subset of recent exposure to rituximab. Anti-spike IgG determined 1–3 months after the second (A) / third (B) dose of COVID-19 vaccine, stratified by the interval between last anti-CD20 regimen and the second / third dose of COVID-19 vaccine.
COVID-19 VACCINATION IMPACTS FUNCTIONAL IMMUNE RESPONSES AND PLASMA PROTEOME IN PLWH

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Background: The impact of COVID-19 infection or COVID-19 vaccination on the immune system of people living with HIV (PLWH) is unclear. We therefore studied the effects of COVID-19 infection or vaccination on functional immune responses and systemic inflammation in PLWH.

Methods: Between 2019 and 2021, 1985 virally suppressed, asymptomatic PLWH were included in the Netherlands in the 2000HIV study (NCT039948350): 1514 participants enrolled after the start of the COVID-19 pandemic were separated into a discovery and validation cohort. PBMCs were incubated with different stimuli for 24 hours: cytokine levels were measured in supernatants. ~3000 targeted plasma proteins were measured with Olink® Explore panel. Past COVID-19 infection was proven when a positive PCR was reported or when serology on samples from inclusion proved positive. Compared were unvaccinated PLWH with and without past COVID-19 infection, and PLWH with or without anti-COVID-19 vaccination excluding those with past COVID-19 infection.

Results: 471 out of 1514 participants were vaccinated (median days since vaccination: 33, IQR 16-66) and 242 had a past COVID-19 infection (median days since +PCR: 137, IQR 56-206). Alcohol, smoking, drug use, BMI, age, latest CD4 count and proportion with viral blips were comparable between groups. Systemic inflammation as assessed by targeted proteomics showed 89 upregulated and 43 downregulated proteins in the vaccinated participants. In contrast, individuals with a past COVID-19 infection display lower levels of inflammation, but less effective cytokine production capacity of its immune system upon stimulation by microbial stimuli, while production of IL-1Ra was increased. In COVID-19 infected PLWH only a reduced production of TNF-α to S. pneumoniae was significant. Vaccinated PLWH also showed upregulation of platelet aggregation pathways.

Conclusion: COVID-19 vaccination in PLWH leads to an increased systemic inflammation, but less effective cytokine production capacity of its immune cells upon microbial stimulation. This pattern is different from that of COVID-19 infection that leads to a decreased inflammatory profile and only minimal effects on cytokine production capacity.

Impact of COVID-19 and Vaccination on Functional Immune Response and Plasma Proteome

372 IMPACT OF GUT MICROBIOTA ON IMMUNOGENICITY TO COVID-19 VACCINES IN PLWH

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Background: Immune responses to SARS-CoV-2 vaccines in people living with HIV (PLWH) have been the focus of several recent studies. As the gut microbiome can influence vaccine immunogenicity, in this study we are the first to investigate whether the baseline gut microbiota can predict immune responses to the BNT162b2 SARS-CoV-2 vaccine in people living with HIV (PLWH) and healthy controls (HC).

Methods: Fecal samples were collected from PLWH (n=68) and HC (n=75) at baseline, prior to the first vaccine dose, to extract DNA for 16S rRNA sequencing. The individuals were part of the COVAXID Clinical trial, where humoral and cellular responses to SARS-CoV-2 vaccine were evaluated on day 35 after the first dose. Comprehensive bioinformatic tools were used for bacterial identification to further reveal the associations between gut microbiota and SARS-CoV-2 antibody, spike CD4+ T cell responses, and clinical parameters such as age, gender, CD4/CD8 ratio, and length of antiretroviral (ART) treatment.

Results: At day 35 post vaccination, HC showed significantly higher spike IgG titers than PLWH (p=0.0001). Interestingly, both phylogenetic and α-diversity were negatively correlated with antibody titers, in the whole cohort and
within groups. Similarly, individuals with low α-diversity had higher levels of spike specific CD4+ T-cell responses. Agathobacter, Lactobacillus, Bacteroides, and Lachnospira were positively correlated with both antibody levels and spike-specific CD4+ T-cell responses while Methanobrevibacter, Marvinirubinanti, Clostridium, and Succinivibrio have a negative one. Within the PLWH group, the gut microbiota taxa associated with CD4+ counts, such as Lachnospira (p=0.002), Oscillibacter (p=0.019) and Flavonifractor (p=0.017), were found to be positively correlated with spike IgG levels. Additionally, the length of ART treatment and CD4/CD8 ratio displayed a positive association with bacterial diversity. Notably, different microbiome profiles and immune status in PLWH, affect their immune responses to vaccination.

**Conclusion:** Our results show potential associations between gut microbiota diversity and spike IgG responses after COVID-19 vaccination. These findings were consistent in the whole cohort, albeit group differences between the microbiome compositions in PLWH and HC were observed. Based on our findings, we propose that microbiome modulation could optimize immunogenicity to SARS-CoV-2 vaccines.

**373 SEQUENTIAL IMMUNIZATION OF UPDATED COVID-19 DNA AND RNA VACCINES IN NONHUMAN PRIMATES**

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**Background:** COVID-19 vaccines that expand immunity against emerging variants of concern (VOC) are needed to protect against ongoing viral evolution. We investigated the impact of boosting nonhuman primates pre-immune to the original WA-1 strain with updated VOC vaccines on the breadth and magnitude of mucosal and systemic antibody (Ab) and T cell (Tc) responses.

**Methods:** Cynomolgus macaques were primed with 2 doses of WA-1 Spike protein encoded by either an IL-12 adjuvanted DNA vaccine administered by gene gun (GG) or a self-amplifying RNA vaccine (repRNA) delivered intramuscularly (IM) with a cationic nanocarrier (LIONTM/IM, HDT Bio) or by GG (Fig 1). A booster dose was administered at week 17 with DNA or repRNA vaccines expressing B.1.351 (Beta) and B.1.617 (Delta) Spike receptor-binding domains (RBDs) fused to influenza HA2 stem domain (SHARP, designed by AIR/WA, USA) or by Gene Gun (WG) followed by a final Beta + Delta + WA-1 SHARP boost at week 34. Blood samples collected from participants (pts) eligible for pre-exposure Mpox vaccination at the time of receiving the first dose (in non-primed) or single dose (in primed) of MVA-BN vaccine (T1) and one month later (T2). MPXV-specific IgG were measured by in-house immunofluorescence assay, using 1:20 as screening dilution; MPXV-specific nAbs by 50% plaque neutralization test (PRNT50, starting dilution 1:10); IFN-γ-producing specific T cells to MVA-BN vaccine, by ELISpot assay. Probability of nAb response in primed vs non-primed at T2 was estimated in pts who had a titre < 10 at T1. McNemar test used to evaluate the overall response, while proportion becoming positive responders at T2 by exposure groups was compared by logistic regression.

**Results:** Average Treatment Effect (ATE) of the difference over T1-T2 by HiSXV was estimated after weighting for age using a linear predictor. Whether the effect of HiSXV on MVA-BN response may vary by HIV infection status was tested by including an interaction parameter in the models.

**Conclusion:** Among the 180 persons self-identified as GBTM MSM, 90 (50%) were historically smallpox vaccinated. Median age was 47 years (IQR 38-54). Within the 86 (48%) PLWH, 81% had a CD4 count of more than 500 cells/mm3. A significant increase in T-cell and nAb response over T1-T2 was observed (Fig.1A-B) and 46 of the 157 who were nAbs non responders at T1 became responders at T2 (McNemar p<0.001). In this subset, the chance of achieving nAb response at T2 was higher in primed vs non-primed [OR=12.2 95% CI (3.4-44.0) after controlling for age]. Results were similar when comparing nAbs in ATE with a mean difference of 0.39 log2 (p=0.03). There was no evidence for a difference in T-cell response according to HiSXV (Fig.1C). There was no evidence that the effect of HiSXV on MVA-BN response varies by HIV status [OR in HIV-neg 1.83 vs HIV-pos 10.7, Interaction p=0.54].

**Conclusion:** The first/single dose of MVA-BN triggers a humoral and cellular response with nAbs response greater in primed vs. non-primed participants independently of age. No evidence that HiSXV effect on nAbs response to MVA-BN differed by HIV status.
**COMPARISON OF SUBCUTANEOUS VERSUS INTRADERMAL ROUTE OF ADMINISTRATION OF MVA VACCINE**

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**Background:** Standard subcutaneous (SC) formulation of 3rd-generation MVA vaccine Ankara (MVA-BN) was replaced by the intradermal (ID) administration route (ad.r), owing to shortages in supplies, based on equivalent reactogenicity and immunogenicity of the SC vs. ID ad.r.

**Methods:** Self-reports of adverse effects following immunisation (AEFIs) were prospectively collected for 7 days from recipients (RCP) of the first MVA-BN dose (T1) as Mpox pre-exposure prophylaxis according to Italian Ministry of Health criteria. Systemic (S-AEFIs: fatigue, myalgia, headache, GI effects, chills) and local (L-AEFIs: redness, swelling, pain) AEFIs were graded as absent (grade 0), mild (1), moderate (2), or severe (3). AEFIs incidence, time of onset, and duration were compared according to ad.r, using chi-square, Fisher, or Mann-Whitney test. C. Potential average change at time T2 post vaccine and Average treatment effect (ATE) from fitting a linear regression model (log scale), weighted for age.

**Results:** 785 MSM with a median age of 38 y (IQR 33-46) received MVA either SC (151;19%) or ID (634;81%). S-AEFIs occurred in 56%, while LSI-AEFIs in 96% estimated after weighting for age using a linear predictor.

**Conclusion:** MVA-BN was generally well tolerated; S-AEFIs were reported more frequently by ID vaccine recipients as well as LSI-AEFI, apart from more frequent local pain after SC. A larger increase in immunological markers was observed with ID vs. SC administration, particularly for IgG and nAb. ID route proved to be safe and immunogenic.

**Figure 1. A. Incidence of SAEFIs and LAEFIs. Comparisons were performed according to the administration route using the chi-square test. B. Titers of MPXV-specific IgG and neutralising antibodies. IgG were measured by in-house immunofluorescence assay, using 1:20 as screening dilution; MPXV-specific neutralising antibodies (nAb) by 50% plaque reduction neutralization test (PRNT50) starting dilution 1:10. Intra-group comparisons were performed with paired t-test; inter-group comparisons by Mann-Whitney test. C. Potential average change at time T2 post vaccine and Average treatment effect (ATE) from fitting a linear regression model (log, scale) weighted for age.

**HUMORAL AND CELLULAR IMMUNE RESPONSE AFTER 3 MONTHS FROM MPOX VIRUS INFECTION**

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**Background:** Immunological signature of Mpox has been described in the early stages of infection. Analysis of its persistence could have implications for decision-making on the need and timing of vaccination in Mpox virus (MPXV) infected subjects. We described the kinetics of humoral and cellular immune response from symptoms onset (FSO) up to 3 months after Mpox.

**Methods:** 9 patients (pts) with Mpox confirmed diagnosis were enrolled from May to July 2022, and blood samples prospectively collected in the early phase of infection (0-20 days) and after 3 months (T90-140). Specific MPXV IgG/IgM and neutralizing antibodies (nAb) titers were measured by immunofluorescence assay and by 50% plaque reduction neutralization test (PRNT50). Interferon-y producing specific T-cells to MVA peptides was assessed by ELISpot assay. In a subgroup of 6 pts, we analyzed the proportion of naive, central memory (CM), effector memory (EM), and terminally differentiated (TEMRA) CD4+ and CD8+ T-cells and their expression of activation and exhaustion markers (CD38/CD57/ PD-1) by flow cytometry. Kinetics of the cellular response were compared with 10 healthy donors matched by sex and age. Kruskal-Wallis and Dunn’s tests, Mann-Whitney and Wilcoxon test were used for statistics, as appropriate.

**Results:** All were MSM with a median age of 39 years (IQR 31-46). 6 were PLWH, all on ART with good viro-immunological status. Only one received smallpox vaccine during childhood.

In all samples, MPXV-specific IgG/IgM have been detected as early as 4 days FSO and peaked during the third week. nAb developed during the second week FSO. At T90-140, IgG and nAb were still detectable, even if significantly lower levels than the peak (Fig1A). MPXV-specific T-cells response significantly increased from T8-11 to T90-140 (Fig1B). Regarding T-cells differentiation profile, a significant expansion of CM and EM cells was observed at T90-140.
377 IMMUNOGENICITY OF MVA-BN VACCINATION WITH HYBRID ADMINISTRATION ROUTE

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**Background:** The limited availability of the Modified vaccina Ankara-Bavarian Nordic (MVA-BN) vaccine during the 2022 human monkeypox (HMPX) outbreak led to the adoption of the dose-sparing regimen of 0.1 ml/dose intradermally administered four weeks apart in two doses. At the time of this decision, the local vaccination campaign was already ongoing, and a part of patients underwent a hybrid scheme consisting of a first subcutaneous administration (SC) followed by an Intradermal one (ID) after 4 weeks.

**Aim of our study** is to assess the neutralization titers induced by a hybrid vaccination schedule.

**Methods:** We enrolled consecutive subjects who attended our Outpatient Clinic for Prevention and Assistance of Sexually Transmitted Infections in the period spanning from August to December 2022. One blood sample was collected at baseline (first dose, SC: T0), week 4 (2nd dose, ID: T1), and week 12 (T2). Virus-neutralizing antibodies titers were estimated by means of Plaque Reduction Neutralization Test (PRNT) against monkeypox virus (MPXV).

**Results:** For 78 patients a T2 sample was available and all of them showed a positive PRNT; of those, 43 patients did not have one of the previous two determinations (n = 37) and had a positive PRNT at T0. The remaining 35 patients were all male, with a median age of 35 years (IQR, 30-39), and among them, 9 people were living with HIV (PLWH) with an optimal viro-immunological control (undetectable HIV RNA and CD4 cells count >500/mmc).

At T1, 4/35 subjects showed no neutralization capacity (of whom 1 PLWH), and the median neutralization titer was 1:20 (IQR, 1:10 – 1:40). At T2, all the patients had a positive PRNT (median neutralization titer was 1:40 (IQR, 1:20 – 1:80)).

Most of the patients increased (21/35, 60%); 5 PLWH) or maintained (4/35, 11.4%); 1 PLWH) the neutralization titer. On the other hand, a 28.6% (10/35); 3 PLWH) showed a reduction of the neutralization titer when compared to T1. No difference in terms of median neutralization titer at T1 [1:20 (IQR 1:10-1:20)] vs 1:20 (IQR 1:20-1:40) and T2 [1:40 (IQR 1:10-1:80) vs 1:40 (IQR 1:20-1:40)] were observed between PLWH and non-PLWH, respectively.

**Conclusion:** A hybrid SC/ID MVA-BN vaccination schedule induced neutralizing antibodies against MPXV at T2. Nevertheless, one out of three subjects showed a decline in the neutralization titer which deserves further investigation.

378 IMMUNE RESPONSES AND VIRAL DYNAMICS AFTER MPOX INFECTION IN THE 2022 OUTBREAK

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**Background:** Human immunity to Monkeypox infection (Mpqox) has not been extensively characterized in people with HIV (PWH). Here, we analyzed antibody responses in outpatients diagnosed with Mpox during the 2022 outbreak in Barcelona, Spain who had been enrolled in the MoViE study (NCT05476744, Suíer et al 2022).

**Methods:** Observational, prospective, multicentre study designed to evaluate time from symptom onset (SO) to viral DNA clearance. A severity score was generated based on the number of local and distal skin lesions and presence of systemic symptoms. Samples from multiple body sites were collected at diagnosis and weekly for one month. Blood was collected from an immune study group (MoViE-Immune) of 100 patients a T2 sample was available and all of them showed a positive PRNT; of those, 43 patients did not have one of the previous two determinations (n = 37) and had a positive PRNT at T0. The remaining 35 patients were all male, with a median age of 35 years (IQR, 30-39), and among them, 9 people were living with HIV (PLWH) with an optimal viro-immunological control (undetectable HIV RNA and CD4 cells count >500/mmc).

At T1, 4/35 subjects showed no neutralization capacity (of whom 1 PLWH), and the median neutralization titer was 1:20 (IQR, 1:10 – 1:40). At T2, all the patients had a positive PRNT (median neutralization titer was 1:40 (IQR, 1:20 – 1:80)).

Most of the patients increased (21/35, 60%); 5 PLWH) or maintained (4/35, 11.4%); 1 PLWH) the neutralization titer. On the other hand, a 28.6% (10/35); 3 PLWH) showed a reduction of the neutralization titer when compared to T1. No difference in terms of median neutralization titer at T1 [1:20 (IQR 1:10-1:20)] vs 1:20 (IQR 1:20-1:40) and T2 [1:40 (IQR 1:10-1:80) vs 1:40 (IQR 1:20-1:40)] were observed between PLWH and non-PLWH, respectively.

**Conclusion:** A hybrid SC/ID MVA-BN vaccination schedule induced neutralizing antibodies against MPXV at T2. Nevertheless, one out of three subjects showed a decline in the neutralization titer which deserves further investigation.
379 NOVEL SEROASSAYS DETECT MPXV-SPECIFIC AND VACCINE-INDUCED ORTHOPOXVIRUS IMMUNITY

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Background: Monkeypox (MPXV) is a zoonotic infection caused by the orthopoxvirus MPXV which is now emerging in a number of countries. A reliable and sensitive assay is needed to detect recent MPXV infection. We describe the development and validation of two novel serological assays for MPXV infection.

Objective: We developed and validated the first MPXV-specific seroassay that can distinguish recent infection from vaccine-induced immunity.

Methods: We developed and validated two serological assays for MPXV infection. The first, the E8L assay, identified MPXV-specific IgG. The second, the B21R assay, identified MPXV-specific IgG and IgM. We performed non-parametric statistical comparisons to distinguish vaccine-induced from vaccine-specific immunity.

Results: In our cohort, vaccinated individuals had high levels of IgG in means of 0.62 vs. 0.056, P < 0.0001 (Fig 1b). B21R IgM was detectable in all samples but low in vaccinated sera (OD 0.27 vs. 0.07, P < 0.05) (Fig 1c).

Conclusion: We developed and validated the first MPXV-specific seroassay which uses the complete B21R peptide, which can distinguish recent infection from vaccination, which in turn was associated with a robust EBL antibody response. Collectively, our assays provide tools for conducting vaccine response and immunosurveillance studies to longitudinally detect immunity to MPXV, determine the true prevalence of MPXV infection and identify asymptomatic community spread.

380 CD103 EXPRESSION ON CD8 T CELLS PREDICTS LONGER TIME REBOUND OF HIV AFTER ATI

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Background: The development of HIV cure strategies depends on our capacity to predict HIV control when antiretroviral therapy (ART) is stopped. Motivated by the recent expansion of noninvasive cell-free DNA (cfDNA)-based cancer diagnostics, we performed multi-dimensional profiling of plasma cfDNA obtained from individuals who maintain HIV suppression following ART cessation termed post-treatment controllers (PTCs), to identify quantitative, genetic, and epigenetic signatures of PTC status.

Methods: cfDNA was extracted from 1 ml of plasma collected prior to ART cessation from 20 PTCs (HIV RNA maintained at <400 copies/ml for >24 weeks post-ART) and 20 non-controllers (NCs) enrolled in ACTG treatment interruption studies, and from 20 uninfected individuals enrolled in the SCOPE cohort. Plasma cfDNA was quantified using the Qubit fluorometer, and whole genome bisulfite sequencing (WGBS) was performed using the IBD Xgen Methyl-Seq kit and the Illumina NovaSeq platform. BSMap, BSbolt, and Metilene software tools were used for sequence alignment, methylation calls, and identification of differentially methylated regions (DMRs), respectively. Statistical analyses and data visualization were performed using the R computing environment.

Results: PTCs exhibited elevated levels of plasma cell-free DNA as compared to NCs (p = 0.007, Mann-Whitney test) and uninfected individuals (p = 0.002) (Fig 1A). Sequencing data revealed higher amounts of circulating mitochondrial DNA (mtDNA) in PTCs as compared to NCs (p = 0.017) and uninfected individuals (p = 0.011) (Fig 1B). WGBS data demonstrated that PTCs had significantly lower
383 PREEXISTING HOST EPIGENETIC STATES ASSOCIATED WITH HIV REBOUND KINETICS

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Background: Viral rebound with variable kinetics consistently occurs in people living with HIV (PLWH) after the discontinuation of ART during analytical treatment interruptions (ATI). In CD4 T-cells, we previously identified candidate host epigenetic DNA methylation (DNAm) states correlating with time to viral rebound during ATI. Whether preexisting host DNAm states in bulk peripheral blood mononuclear cells (PBMCs) of PLWH participating in diverse curative interventions can reveal novel preexisting host epigenetic signatures related to time to viral rebound remains understudied.

Methods: Genome-wide DNA methylation profiling of PBMCs was obtained at study entry from 26 male PLWH on ART who participated in an ATI following combination interventions designed for eradicating residual plasma viremia and decreasing HIV reservoirs (NCT02961829). 15 early rebounding participants ranged in time to viral rebound from 14-24 days, 10 late rebounding participants from 33-102 days, and one exceptional participant (São Paulo Patient) rebounded at 511 days. Differentially methylated loci (DML) associated with the rebound group were identified by comparing early and late groups at a mean difference in methylation greater than 5% (Δβ-value) with FDR correction.

Results: Pre-ATI total HIV DNA at baseline and CD4 count did not significantly associate with time to viral rebound. At baseline study entry visit prior to intervention, we identified 2,694 DML (Δβ-value > 0.05; P < 0.05) comparing early compared to late rebounding participants (Fig. 1). These loci were enriched at intergenic regulatory regions and upstream of CpG islands in the human genome (P=0.001). Gene ontology and pathway enrichment analyses revealed DNAm differences at genes involved in the MHC class II protein complex, interferon-gamma-mediated signaling pathway, platelet activation, and T helper cell differentiation. Among the top DML associated with rebound group were genes including ADAMTS20 involved in HIV life cycle, antiviral protein against HIV, epigenetic regulator of plasmacytoid dendritic cells CX3CR1, and chemokine gene PF4AV. DNAm states of PRSS30, MX2, ZNF529, and ZNF583 significantly associated with viral load post-ATI.

Conclusion: Fixed epigenetic features of innate and adaptive immune cells in PLWH may predetermine varying viral rebound kinetics during ATI despite multimodal therapies. These findings also suggest that an optimal host epigenetic landscape may exist to control HIV expression and silencing. Correlogram plot of time to viral rebound, viral load post-ATI, HIV DNA pre-ATI, CD4 count pre-ATI, and pre-ATI DNAm states of host genes.
PARTICIPANT EXPERIENCES IN A CURE-DIRECTED LONG-TERM TREATMENT INTERRUPTION
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Background: Most HIV cure-directed clinical trials require an analytical treatment interruption (ATI) to test the efficacy of interventions aimed at keeping HIV suppressed in the absence of antiretroviral treatment (ART). Little is known about how people with HIV (PWH) articulate or feel about having experienced extended ATIs.

Methods: From April – August 2022, we conducted two sequential qualitative interviews with participants in the BEAT-HIV-02 (NCT03588715) HIV cure-directed trial testing a combination of immunotherapy (peg-IFN-α2b) with two broadly neutralizing antibodies (3BNC117 and 10-1074) given for 26 weeks after ATI interruption. Immunotherapy was followed by up to an additional 24-week follow-up ATI until ART re-start criteria was met. Interviews took place at study baseline and immediately after completion of study ATI when re-start ART criteria were met. Interviews elicited participant experiences during the ATI and the trial in general. Interviews were recorded, transcribed, and analyzed using directed content analysis.

Results: In total, 13 PWH, 77% male, 77% Black, completed sequential interviews. The mean ATI was 38 weeks in duration. Participants viewed the ATI as positive because they appreciated a respite from daily medication. Some reported increased self-confidence when their counts remained low during the ATI, before viral rebound. However, when viral counts rose, some expressed feelings of fear, frustration, anger and despair. Three expressed disappointment that they were not cured of their HIV. Rising viral loads led some to feel a sense of failure. All participants reported a positive and trusting relationship with the clinical trial team. Reciprocal respectful relationships between participants and study staff were noted as helping to mitigate participants’ safety concerns.

Conclusion: Our socio-behavioral study identifies key points for intervention and participant support during HIV cure-directed studies including an extended ATI. Managing expectations, focusing on participants’ contributions, and providing support to reduce feelings of having failed the research team and/or the HIV community following viral rebound should be part of study design. Continued efforts to understand how PWH experience ATIs will improve future designs of HIV cure-directed clinical trials.

Key Themes and Representative Quotes

| ATI sequence was positive | “It’s hard to explain how good it feels not to take one pill. Every day I went without it, just made it feel so good... I am optimistic.” |
| ATI was less positive | “My viral load jumped up high... it had me a little scared, because I had never seen my numbers in that range since I’ve been diagnosed.” |
| Research team conferred a sense of safety and trust that mitigated the sense of risk | “It’s like being in a safe place and that’s what makes them feel like, I’m not just a guinea pig or not just a number and I want to be part of the cure.” |

PLASMA CD33 LEVEL IS A MARKER OF VIRUS CONTROL POST-KICK-AND-KILL CURE INTERVENTION
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Background: HIV cure strategies will require the elimination of latently HIV infected cells from all sites of the viral reservoir, including central nervous system. Latency reversing agents (LRA) that can reach all these sites may thus be needed. The BCN02 trial (NCT02616874) combined the HIVancon T-cell vaccine with the latency reversing agent romidepsin (RMD), a LRA that also has been linked to beneficial effects in neurological diseases. To identify biomarkers associated with virus control during monitored antiretroviral pause (MAP), longitudinal proteomics screenings were conducted in plasma from BCN02 participants.

Methods: Plasma proteomes of longitudinal samples from 11 BCN02 participants, including 8 MAP non-controllers (MAP-NC, viral loads >2000 copies/ml/ <4 weeks) and 3 MAP-controllers (MAP-C, viral load < 2000 copies/ml for >32 weeks) were determined. Integration data analysis (viral load, proviral levels, and neurocognitive assessments) was performed to identify candidates. For validation, untreated chronically HIV-infected individuals (n=96) with different levels of virus control were included. Finally, in vitro viral replication assays and proviral quantification targeting CD33 in PHA-stimulated T-cells and macrophages derived monocytes were performed.

Results: During the BCN02 trial, plasma proteomes changed longitudinally, with most changes observed after RMD infusions and during MAP and significant differences between MAP-C and MAP-NC were observed already before the initiation of the intervention. CD33 protein was uniquely increased upon RMD administration and maintained during MAP and allowed to discriminate between MAP-C and MAP-NC, even when assessed before RMD treatment. CD33 plasma levels were positively associated with viral load and proviral levels in the BCN02 trial. Validation untreated chronically HIV-infected individuals showed higher plasma levels of CD33 associated with reduced virus control. While neurocognitive assessments did not correlate with CD33 plasma levels in the BCN02 trial, positive correlations between CD33 protein and neurofilament light protein were observed after RMD administration and during MAP. In vitro targeting of CD33 showed reduced HIV replication and proviral levels, suggesting an important role of CD33 in the HIV life cycle.

Conclusion: This study identifies CD33 as key factor associated with virus control post Kick-and-Kill intervention and in natural HIV infection. Targeting CD33 may be considered for future HIV therapeutic cure strategies.

COMPREHENSIVE ANALYSIS OF VIRAL RESERVOIRS IN HIV-INFECTED ELITE CONTROLLERS
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Background: Antiretroviral therapy (ART) has greatly improved health outcomes in people living with the human immunodeficiency virus (HIV), but the persistence of viral reservoirs remains a barrier to viral eradication. Given that HIV-infected elite controllers (ECs) can naturally suppress plasma viremia to undetectable levels without ART, systematic analyses of their viral reservoirs may be key to understanding how to achieve ART-free virologic control in infected individuals. Previous studies have found that ECs have relatively small HIV DNA burdens, potent HIV-specific immunity, and integration patterns that contribute to long-term HIV control. However, few have compared the inducibility of infectious HIV between ECs and chronically infected, anergic individuals receiving ART (CAs).

Methods: HIV reservoirs of 9 ECs and 22 CAs were compared by measuring intact proviral DNA, defective DNA, and cell-associated HIV RNA in CD4+ T cells with PCR-based assays. Cellular assays were used to measure inducible virus-associated HIV RNA and replication-competent virus. HIV-specific immune responses were measured following stimulation with HIV Gag peptides. Full-length (NFL) sequencing of proviral DNA in effector memory CD4+ T cells was performed. Statistical analysis was performed with the Mann-Whitney test.

Results: Levels of intact proviral DNA, defective DNA, and cell-associated HIV RNA were lower in ECs compared to CAs. However, the levels of inducible and replication-competent virus were similar between both groups, and ECs had higher frequencies of HIV-specific CD8+ T cells, suggesting the presence of viral antigen expression. Of three ECs with extraordinarily low inducible/infectious viral reservoirs, one had high copy numbers of intact proviral DNA by ICPA, but NFL sequencing revealed that all clonotypes were defective.

Conclusion: We demonstrated that ECs have lower levels of intact proviral DNA and cell-associated HIV RNA but comparable levels of inducible and replication-
competent virus compared to CAs. The discordance observed between the PCR- and cellular-based assays may suggest that many identified intact proviral DNA in our CA group are, to a certain extent, replication-defective, as seen in one EC. Thus, although PCR-based methods provide insight into the composition of viral reservoirs, it is essential to perform assays that detect replication-competent virus in certain populations of HIV-infected individuals, such as ECs, to accurately estimate the size of the viral reservoir.

Analyses of HIV reservoirs in elite controllers and chronically infected arieveric study participants

387 PREDICTORS OF HIV REBOUND AFTER INTERRUPTION OF ART STARTED DURING PRIMARY INFECTION
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Background: Understanding factors that affect timing of viral rebound after antiretroviral treatment (ART) interruption will accelerate efforts toward inducing sustained HIV remission. Here, we evaluated whether HIV DNA size, activity, and molecular diversity as well as peripheral T cell phenotypes prior to treatment interruption predict time to HIV rebound in individuals interrupting ART initiated during primary infection.

Methods: The Zurich Primary HIV Infection Cohort (ZPHI) enrolled people with HIV (PWH) who started ART during primary infection and interrupted therapy after a median of 18 months of viral suppression. We selected stored cross-sectional samples (pre-ART interruption) from 70 ZPHI participants for this study. Using flow cytometry, we evaluated frequencies of T cell maturation (CD27−CD45RA+CD69+/−TIGIT+, proliferation (Ki67+), degranulation/cytotoxicity (CD107a+) and regulatory CD4+ T cells (CD25+FoxP3+) on a subset of 38 individuals. We characterized quantitative and qualitative changes in SIV-infected cell populations following ART initiation by providing insights into the cells that enter the long-term reservoir. Recently, White et al. explored the decay kinetics of infected CD4+ T cells in people living with HIV-1 (PLWH) on ART and found that intact genomes decayed with biphasic kinetics characterized by a rapid initial phase, and a slower 2nd phase that was faster than the decay in PLWH on long-term ART. The decay of SIV-infected cells in macaques on ART has not been described. Analogous studies using the SIV/non-human primate model offer an opportunity for insights difficult to obtain in PLWH.

Methods: We characterized quantitative and qualitative changes in SIV-infected CD4+ T cells for 4 years following ART initiation. We used digital droplet PCR to compare the decay of intact and defective proviruses, as well as single-genome sequencing (SGS) of env from plasma virus and proviral DNA to look for qualitative changes during ART.

Results: By following treated macaques for 4 years, we observed both early and late phase dynamics of the latent reservoir. We found that the decay kinetics of cells harboring intact SIV proviruses are similar to those reported for HIV-1, characterized by multiphasic decay and a final phase representing stable persistence of a latent reservoir. env SGS revealed that ART potently stops viral evolution and that the composition of infected cell populations changes during ART. We found that the average pairwise distance from the stock decreased over time on ART, reflecting decay of recently infected cells. Ancestral variants with fewer mutations that had been archived in the latent reservoir were not present in the plasma at ART initiation and became prominent over time on treatment.

Conclusion: These data provide a baseline decay rate for SIV-infected cells in macaques on ART, which is critical to accurately evaluate cure strategies aimed at the latent reservoir. Our results show that variants enter the reservoir throughout untreated infection and not just at ART initiation. The population of SIV-infected CD4+ T cells is dynamic and changes both quantitatively and qualitatively for several years following ART initiation. These data provide a framework for evaluating and interpreting intervention trials utilizing the SIV/NHP model.

Conclusion: We found viral and immune factors associated with delayed rebound of HIV RNA after ART interruption in PWH starting ART during early infection. Our results suggest that a combination approach will be necessary to boost viral control, including early ART start to limit viral diversity and additional interventions to reduce or reverse T cell activation and degranulation/cytotoxicity.

Table 1: Factors significantly predictive of time to viral rebound

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% confidence limits</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminally Differentiated CD4+ T Cells expressing CD38 and HLA-DR</td>
<td>0.0363</td>
<td>0.0080</td>
<td>0.0190</td>
<td>0.0517</td>
</tr>
<tr>
<td>Effecter memory CD4+ T Cells expressing CD38 and HLA-DR</td>
<td>0.0111</td>
<td>0.0024</td>
<td>0.0067</td>
<td>0.0165</td>
</tr>
<tr>
<td>Central Memory CD4+ T Cells expressing CD38/71</td>
<td>0.0353</td>
<td>0.0131</td>
<td>0.0003</td>
<td>0.0003</td>
</tr>
<tr>
<td>Terminally Differentiated CD8+ T Cells expressing CD38</td>
<td>0.0154</td>
<td>0.0075</td>
<td>0.0022</td>
<td>0.0078</td>
</tr>
<tr>
<td>Central memory CD8+ T Cells expressing CD38/71</td>
<td>0.1810</td>
<td>0.0082</td>
<td>0.0032</td>
<td>0.0072</td>
</tr>
<tr>
<td>Mean molecular diversity HIV DNA/106 bp</td>
<td>0.0126</td>
<td>0.0099</td>
<td>0.0070</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

Legend: Estimate = the estimated effect size of the factors on the hazard of viral rebound. An estimated effect size one or more than one indicates that as the factor increases, the hazard of viral rebound increases. Standard Error = the standard error of the estimate. 95% Confidence Limits = the lower and upper 95% confidence intervals for the estimate. The lower bound = Estimate + 1.96Standard Error and the upper bound = Estimate − 1.96Standard Error. p-value = the p-value from testing the significance of the effects. A p-value < 0.05 indicates a significant effect of the factor on the hazard of viral rebound.
389 PRESENTATION OF COGNATE ANTIGENS BY DENDRITIC CELLS CAUSES STOCHASTIC HIV EXPRESSION

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Background: Encounter with cognate antigens (Ag) is a major cause of proliferation and persistence of HIV-infected CD4+ T cells. Understanding to which extent physiological T cell activation leads to latency reversal is key for cure efforts. However, due to the low frequency of HIV-infected Ag-reactive cells, previous studies failed to compare HIV reactivation mediated by Ag recognition versus global T cell activators.

Methods: CD8-depleted PBMCs from 10 PLWH on ART were stimulated with either CMV or HIV Gag Ag. Reactive cells (CD40L+) were isolated and expanded for 10–14 days. Expanded pools were characterized by TCR repertoire, total and intact HIV DNA, and proviral sequencing. After resting for 3 days, cells were re-stimulated with either PMA/Io, anti-CD3/CD28, or autologous dendritic cells (DCs) pulsed with cognate or an unrelated Ag (KLH). We measured T cell activation by CD40L and CD69 expression, and profiled HIV RNA by bulk and limiting dilution digital PCR and sequencing.

Results: Our approach allowed us to isolate rare Ag-reactive CD4+ T cells (range 0.5–2%) and expand them while preserving their overall TCR repertoire. The frequency and composition of proviruses in the expanded pools were highly variable across participants. Although most HIV genomes were defective, we detected intact proviruses in 2/5 and 5/5 participants’ CMV or Gag reactive cells, respectively. Upon re-stimulation with DCs, only cognate Ag caused significant T cell activation (CMV 69.5%, Gag 64.4%, KLH 4.08%, p < 0.0001). Although Ag stimulation increased HIV expression compared to baseline (p = 0.004), HIV reactivation was variable (fold change 0.83–50.56). While CMV-reactive pools from 2 participants showed high induction of HIV expression (46- and 50-fold), 4/10 Ag pools exhibited little HIV RNA increase compared to baseline (fold change 0.8–4). Conversely, treatment with PMA/Io or anti-CD3/CD28 caused significant HIV RNA production across all participants (fold change mean 13.5 and 6.5, respectively). Limiting dilution RNA assays showed similar breadth of proviruses induced across conditions, but only strong stimulation (PMA/Io) lead to high HIV RNA-producing cells (>10^4 cpsi/cell).

Conclusion: These results suggest that there are quantitative and qualitative differences in cellular and HIV transcriptional profiles when CD4+ T cells encounter their cognate Ag compared to global T cell activators. Our work suggests that, for some proviruses, physiological T cell activation is insufficient to fully reverse HIV latency.

390 STABILITY AND INSTABILITY OF THE CELLULAR HIV RESERVOIR AFTER REBOUND DURING ART

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Background: The complex pool of infected cells that comprise the HIV reservoir can be distributed amongst CD4+ T subsets with varied functional and compartmental characteristics. Recent studies during treatment interruption in passive immunotherapy trials have demonstrated that reservoir reseeding can coincide with viral rebound. However, whether reseeding is associated with compartmental characteristics remains uncertain. Recent studies during treatment interruption can coincide with viral rebound. However, whether reseeding is associated with compartmental characteristics remains uncertain.

Methods: We profiled the reservoir from three participants of the clinical trial (ACTG A5340) who experienced viral rebound after receiving the broadly neutralizing antibody VRC01 during analytical treatment interruption (ATI). Pre-ATI and post-ATI blood samples were collected while viral load was fully suppressed. We applied viral single-cell Assay for Transposase Accessible Chromatin with Select Antigen Profiling by sequencing (ASAPseq) to identify HIV+ cells using accessible proviral DNA and their coordinate cell surface markers. Peripheral blood memory CD4+ T cells were enriched by bead separation and labeled with oligo-tagged antibodies for generation of viral ASAPseq libraries. Reads were processed using our custom pipeline which included alignments to consensus and autologous viral sequences.

Results: We profiled 136997 memory CD4+ T cells with viral ASAPseq, of which 205 cells (0.13%) were detected as HIV+. After clustering and annotating with epigenetic and surface antigen data, we compared the phenotypes between the pre-ATI and post-ATI timepoints for each individual. In one individual with a low viral rebound (as determined by area under the curve; AUC) during ATI, phenotypic composition of HIV+ cells was maintained. In contrast, the other individuals with higher viral load rebound had greater disruption of the phenotypic composition of HIV+ cells. Reservoir modulation was specifically associated with the emergence of recently activated Tm/Tcm cells at the post-ATI timepoint.

Conclusion: Our observations suggest that the extent of viral rebound AUC is associated with greater changes in reservoir phenotype. These results suggest that incomplete viral suppression during clinical trial interventions can lead to diversification of the cellular phenotypes found in the HIV reservoir.

391 LACK OF DETECTABLE ONGOING REPLICATION ON ART IN SIV-INFECTED RHESUS MACAQUES

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Background: The capacity of HIV-1 to replicate during antiretroviral therapy (ART) remains controversial. Limitations of clinical sampling and the high background level of genetic diversity in most persons living with HIV make it challenging to detect additional substitutions on ART. To gain greater sensitivity to detect even small changes to the virus population on ART, we used an NHP model, which allows precise control over the level of pre-ART evolution and subsequent changes to the viral population.

Methods: We infected 21 rhesus macaques with the barcoded virus SIVmac239M to compare near-full-length (nFL) SIV DNA single genome sequences from PBMCs (and in some cases lymph nodes and spleen) obtained near the time of ART initiation and those present after long-term ART. Animals started ART between 10–27 days post infection (dpi) and were treated for 285–977 dpi. We obtained 25–200 intact nFL sequences per animal. We then assessed whether the viral populations differed significantly between the two time points in (i) genetic diversity, (ii) population structure, and (iii) genetic divergence from the founder.

Results: We found no evidence for replication on ART for any of the animals. The median average pairwise distance did not differ significantly between baseline and long-term ART samples (0.0155 vs 0.015%; p-value = 0.23; two-tailed Wilcoxon-signed rank test). The probability that sequences from the two samples came from different populations was not statistically significant in any animal (test for panmixia). Finally, the regression slope of p-distance over time did not differ significantly from zero in any animal. Neighbor-joining trees were also consistent with a lack of viral evolution, displaying no clustering of sequences from different time points, or longer branches associated with sequences obtained from long-term ART.

Conclusion: Overall, these data are consistent with the most recent papers in the field, which did not find evidence for evolution of HIV on ART. We cannot exclude the possibility of low-level ongoing viral replication not detectable in our study despite intensive sampling. For example, computational modeling suggests that ongoing replication in a theoretical sanctuary site could lead to viral evolution while maintaining the level of plasma viremia below the limit of detection. However, the potential contribution of such a small replicating population to the rebound–competent reservoir or as a source of drug resistance escape mutations would be limited.

392 SLOWING OR REVERSAL OF DECAY OF INTACT PROVIRUSES OVER 2 DECADES OF SUPPRESSIVE ART


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Background: The intact proviral DNA assay is a measure of the replication-competent HIV reservoir. Little is known about the decay patterns of intact proviral DNA (IPD) in people with HIV (PWH) during very long-term (15-20 yr) suppressive antiretroviral therapy (ART).

Methods: Participants in ACTG AS231 with chronic HIV and documented suppression of viremia (HIV RNA < 50 copies/mL) for >15 yr of ART had measurements on blood samples of IPD, 5' or 3' defective proviral DNA, and
Results: Fourteen participants (5 female) were evaluated longitudinally from ART yr 1 to ART yr 17-23 (median 20 yr of ART; 8-10 timepoints). Median pre-ART plasma HIV RNA 4.2 log_{10} c/ml; median pre-ART CD4 cell count 373/mm^3. At yr 1 of ART, median intact proviruses were 204 copies/million CD4 cells; median (Q1, Q3) intact provirus percentage (intact/total) was 66% (41, 83). By the late time point (median 20 yr on ART), intact provirus percentage had fallen to 7% (4, 10), reflecting selective decay of intact proviruses (decay of intact proviruses was 13-fold compared with 3-fold for total proviruses). Over the two decades on ART, 5 participants had biphasic decay in IPD levels, 3 participants had biphasic decay with a second phase plateau (slope effectively zero), and 2 participants showed evidence of increased IPD levels during the second decade of ART (see Figure). The inflection or transition of decay occurred at a median of 5 yr after ART initiation (range 2-13 yr). The median IPD first phase half-life was 1 yr (n=10), whereas the median IPD second phase half-life was >25 yr (n=8). (For the 2 participants with late IPD increases, second phase half-life was undefined.) In the 4 other participants, there was a variable pattern of IPD decay, perhaps in part due to fewer cells assayed or low IPD levels.

Conclusion: In PWH on very long-term ART, three patterns of IPD decay were revealed despite continual suppression of viremia: 1) biphasic decline with markedly slower second phase decline; 2) initial decline that transitions to a zero slope plateau; and 3) initial decline followed by late increases in IPD. The mechanisms of markedly slower second phase decay or reversal are uncertain but may include the inability to clear cells with intact but transcriptionally silent proviruses and clonal expansion of cells with intact proviruses.

Examples of decay patterns in intact proviral DNA levels for participants on long-term ART. Five participants had a similar pattern as shown on left (A), with slowing decay of intact proviruses during the second decade of ART. Three participants showed plateauing of decay (slope effectively zero) (B) and two participants had patterns of late increases (C).

**Differential Transcription Levels of HIV-1 Full-Length and Highly Deleted Proviruses**

**Human Galectin-9 Promotes the Expansion of HIV Reservoirs in Vivo in Humanized Mice**

**394 DIFFERENTIAL TRANSCRIPTION LEVELS OF HIV-1 FULL-LENGTH AND HIGHLY DELETED PROVIRUSES**

**395 HUMAN GALECTIN-9 PROMOTES THE EXPANSION OF HIV RESERVOIRS IN VIVO IN HUMANIZED MICE**

**394**

**HUMAN GALECTIN-9 PROMOTES THE EXPANSION OF HIV RESERVOIRS IN VIVO IN HUMANIZED MICE**

Zhe Yuan, Leila Giron, Colin Hart, Kwasi Gyampoh, Jane Koshy, Kai Ying Hong, Toshiro Niki, Thomas Premaceaux, Lis homwda Mdloulo, Luis J. Montaner, Mohamed Abdel-Mohsen

**Background:** The human endogenous β-galactoside-binding protein Galectin-9 (Gal-9) reactivates latently HIV-infected cells, which may allow for immune-mediated clearance of these cells. However, Gal-9 also activates T cell Receptor (TCR) signaling pathways, which could negatively affect HIV persistence by promoting T cell expansion and chronic immune activation and exhaustion. This potential “immunomodulatory” effect of Gal-9 during HIV infection raises the question of the overall impact of Gal-9 on HIV persistence in vivo.

**Methods:** We used the BLT (bone marrow, liver, thymus) humanized mouse model to evaluate the overall impact of Gal-9 on HIV persistence in vivo during antiretroviral therapy (ART). Two independent cohorts of BLT mice with high human immune reconstitution were infected with HIV, placed on ART, and then treated with either recombinant human Gal-9 or PBS during ART suppression. Plasma viral loads and levels of tissue-associated HIV DNA and RNA were measured by qPCR. Markers of T cell activation/exhaustion were measured by flow cytometry, and plasma markers of inflammation were measured by multiplex cytokine arrays.

**Results:** Gal-9 treatment was tolerable in ART-suppressed humanized mice and did not significantly induce plasma markers of inflammation or T cell markers of activation or exhaustion. However, Gal-9 treatment during ART significantly increased levels of tissue-associated HIV DNA and RNA compared to controls (P=0.0007 and P=0.011, respectively, for cohort 1 and P=0.002 and P=0.005, respectively, for cohort II).

**Conclusion:** Our study reveals a detrimental effect of Gal-9 on HIV persistence in vivo, suggesting instead for blockade of Gal-9 interactions as a strategy for reservoir reduction in PLWH.
**HIV-1 CLADE C RESERVOIR CHARACTERISTICS IN EARLY AND CHRONIC TREATED INFECTION**

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**Background:** Persisting HIV reservoir viruses in resting CD4+ T cells and other cellular subsets remains a barrier to curative efforts. Early antiretroviral treatment (ART) has been shown to enable post-treatment viral control in some cases. We hypothesized that extremely early ART initiation will affect the size, decay dynamics and landscape characteristics of the HIV-1 subtype C viral reservoirs.

**Methods:** We studied 35 women from the FRESH cohort in Durban, South Africa diagnosed with hyperacutie HIV infection by twice weekly testing for HIV-1 RNA. Study participants were divided into two groups where, 11 started ART at a median of 450 (297-1203) days post onset of viraemia (DPOV), while 24 started ART at a median of 1 (1-3) DPOV. We used PCR to measure total HIV DNA by ddPCR and sequence viral genomes by full length individual proviral sequencing (FLP-Seq) from detection of HIV up to 1 year post treatment.

**Results:** ART in hyperacute infection reduced peak viraemia compared to untreated infection (p = 0.0001), but there was no difference in total HIV DNA measured contemporaneously (p = 0.104). There was a steady decline of total HIV DNA in the early treated group over 1 year that was not observed in the late treated group (p = 0.0004). Total HIV DNA after 1 year of treatment was lower in the early treated compared to the late treated group (p = 0.02). We generated 697 single viral genome sequences. There was a difference in the longitudinal proviral genetic landscape over 1 year between untreated, late treated and early treated infection, where the relative contribution of intact genomes to the total pool of HIV DNA after 1 year was higher in untreated infection (31%) compared to late treated (14%) and early treated infection (8%). Treatment initiated in both late and early infection resulted in a more rapid decay of intact (T1/2 =2 months and T1/2 =0.75 months versus defective (T1/2 =25 months and T1/2 =8.54 months) viral genomes. However, intact genomes were still present with other interventional strategies.

**Conclusion:** Extremely early ART initiation in subtype C HIV-1 was associated with a more rapid decay of intact viral genomes, decreased genetic complexity and immune escape which could accelerate reservoir clearance when combined with other interventional strategies.

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**PERSISTANCE OF INDUCIBLE REPLICAATION-DEPENDENT HIV-1 AFTER LONG-TERM ART**

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**Background:** Latent HIV-1 persists in a population of resting CD4+ T cells (rCD4s) harboring a transcriptionally silent provirus. It was previously shown that this latent reservoir decays slowly in individuals on antiretroviral therapy (ART) over the first seven years of therapy with a half-life of 44 months (Siliciano, Nat Med, 2003). However, it is unclear if the latent reservoir continues to decay in people on very long-term ART.

**Methods:** We recruited 11 people living with HIV (PLWH) who had been on suppressive ART for at least 20 years. To determine reservoir size and the frequency of inducible, replication-competent HIV-1, we performed quantitative viral outgrowth assays (QVOAs) with purified rCD4s. Wells scored positive for viral outgrowth were subjected to RNA extraction and full length env sequencing to explore the clonality of the reservoir. Additionally, we extracted genomic DNA from rCD4s to quantify the proportion of intact and defective HIV proviruses using the intact proviral DNA assay (IPDA).

**Results:** Viral outgrowth was detected in all study participants in the QVOA. The frequency of rCD4s harboring inducible, replication competent HIV ranged from 0.03-16.2 infectious units per million (IUPM) and was highly correlated with the number of intact proviruses detected in the IPDA. Intact proviruses detected by the IPDA ranged from 2.8-168.3 copies per million cells and comprised < 10% of the total number of proviruses detected using this assay. Full-length env sequencing results suggest a trend in several individuals towards a high proportion of identical sequences.

**Conclusion:** In this study, we demonstrated that the latent reservoir in PLWH on ART for more than 20 years harbors inducible, replication-competent proviruses. The range of IUPM values measured in this study are similar to those measured previously, indicating that over a very long time interval, the reservoir does not decay significantly. This is likely because decay processes are counterbalanced by infected cell proliferation. Full-length env sequences exhibited limited diversity in some individuals, implying that expanded T cell clones harboring identical proviruses contribute to long-term reservoir persistence. Ultimately, these data provide evidence that continued ART is necessary even following 20+ years of ART.

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**IN-DEPTH TRANSCRIPTOMIC ANALYSIS OF THE TRANSLATION-DEPENDENT HIV-1 RESERVOIR**

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**Background:** We previously described a Tat mimetic (Tat #1) that induces HIV reactivation in a higher fraction of latently-infected cells compared to PMA/ionomycin, without impacting cell viability nor modifying the transcriptome of the cells. Here we took advantage of these unique properties to study viral and cellular transcripts in p24+ cells following reactivation.

**Methods:** CD4 T cells from 5 ART-treated individuals were treated for 48h with Tat #1. A total of 108 p24+ cells and 109 p24- cells were single-cell sorted and subjected to single-cell RNA sequencing by Smart-seq2. Transcriptomic differences between p24+ and p24- cells were validated at the protein level using flow cytometry.

**Results:** Among p24+ cells, 6.7% of the detected reads mapped to the HIV reference genome HXB2, while HIV reads were not detected in p24- cells. Levels of HIV transcripts positively correlated with the intracellular p24 production per cell. Following de novo assembly of HIV transcriptional reads, complete reconstitution of the viral genome could be obtained in 36% of the p24+ cells, independently of the HIV subtype. Proviruses with a defective major splice donor (MSD) site and cryptic donor (CD) site utilized alternative splice sites up- and/or downstream of the MSD, highlighting the plasticity of MSD/CD defective proviruses. Compared to p24- cells, p24+ cells significantly expressed higher levels of a previously undescribed long non-coding (lnc) RNA, SOD1P3, CCL5 and GZMA, while expressing lower levels of ATG10 and IL7R. Flow cytometry analyses confirmed that p24+ cells expressed higher levels of CCL5 and GZMA proteins while expressing lower levels of IL7R and GZMB proteins compared to p24- cells.
In vitro infection of primary CD4 T cells with the virus strain 89.6 induced a strong upregulation of the IncRNA expression. Furthermore, pre-treatment of primary CD4 T cells with three different antisense oligonucleotides targeting the IncRNA prior in vitro infection resulted in the efficient silencing of the IncRNA while reducing the percentage of p24+ cells compared to the non-treated control (≈3-fold, n=3 donors).

**Conclusion:** Using single-cell RNA sequencing, we unravelled alternative splicing mechanisms and showed that p24+ cells display a distinct transcriptional signature compared to non-infected cells. We identified a IncRNA as preferentially expressed by p24+ cells and provided evidence this IncRNA may play a role in regulating HIV gene expression.

### 398 CTL RESPONSES ARE NOT ASSOCIATED WITH DECAY OF INTACT PROVIRUSES OR HIV RNA ON ART

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**Background:** HIV-specific T-cells targeting Nef but not other HIV proteins are associated with levels of total HIV DNA and cell-associated (CA)-RNA that persist on ART, suggesting ongoing Nef antigenic stimulation. It is not known, however, if HIV-specific T-cells impact either proviral persistence or expression. We hypothesized that decay of intact proviral DNA and CA-RNA levels on ART would be associated with cytotoxic T-cell (CTL) responses.

**Methods:** 49 participants from the ACTG A5321 cohort on suppressive ART (plasma HIV RNA < 40 copies/mL) were studied at weeks 24 and 168 post-entry (median 7 years on ART at entry). HIV DNA and CA-RNA were measured by droplet digital PCR (IPIPOA for DNA, S' unspliced and 3' total poly(A) for RNA). T-cell responses were measured by IFN-γ and granzyme B (GrB) ELISPOT to each HIV gene product (Gag, Env, Pol, Nef, Tat, Rev, and summed HIV). Non-parametric statistics were used to evaluate associations and to compare time points.

**Results:** S' unsplashed CA-RNA decreased significantly from week 24 to 168 (p = 0.001), and decline in intact (p = 0.053) but not defective (p = 0.22) HIV DNA approached significance. CA-RNA levels at weeks 24 and 168, and changes from 24 to 168 weeks were not found associated with CA-RNA levels or changes over time. There were no apparent associations between measures of HIV-specific T-cell responses (both IFN-γ-producing and GrB-producing) with the changes in intact or defective proviruses, nor with the changes in CA-RNA levels — including after controlling for time on ART, pre-ART viral load, and pre-ART CD4 count. As examples, the correlations between magnitudes of IFN-γ-producing Nef-specific responses and changes in intact HIV (r = 0.11, p = 0.61) and S' CA-RNA (r = 0.06, p = 0.71), or GrB-producing Nef-specific responses and changes in intact HIV (r = 0.10, p = 0.66) and S' CA-RNA (r = 0.14, p = 0.37), were small and not significant.

**Conclusion:** While both intact proviral DNA and CA-RNA levels (S' unsplashed) decayed over the 144-week period, contrary to our primary hypothesis no associations were observed between decay of intact HIV DNA or CA-RNA with HIV-specific T-cell responses, including with cytotoxic function (GrB) and despite controlling for time on ART. These findings suggest either a limited role for CTLs in reservoir decay after multiple years of suppressive ART, or that other unmeasured parameters are important, such as variation in susceptibility of reservoir cells to CTL-mediated killing.

### 399 TSCM/TCM-ENRICHED ANTI-HIV duoCAR-T CELLS EXERT POTENT HIV CONTROL


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**Background:** HIV-specific T-cells targeting Nef but not other HIV proteins are candidates to functionally cure HIV infection in people with HIV (PWH). Translating such therapeutic candidates successfully into PWH will require persistence and therapeutic efficacy. Here, we hypothesized that early-memory enriched anti-HIV duoCAR-T cells generated using a GMP-compliant CAR-T cell manufacturing process would exert potent HIV control in humanized mice with productive HIV-1 infection.

**Methods:** To test this hypothesis, we developed an 8-day CAR-T cell manufacturing process and profiled the T cell differentiation state of pre-infusion anti-HIV duoCAR-T cell products using multiparametric flow cytometry and CyTOF analyses. The therapeutic efficacy of early-memory enriched anti-HIV duoCAR-T cells was evaluated in a humanized NSG mouse model of intrasplenic HIV-1 infection (hu-spl-PBMC-NSG).

**Results:** CyTOF analyses of pre-infusion duoCAR-T cells revealed a unique early-memory phenotype composed predominantly of CCR7+ stem cell-like/central memory T cells (Teff/Tcm) with effector-like characteristics. We show that peripheral-injected Tcm/Tcm enriched anti-HIV duoCAR-T cells localized to the site of active HIV infection in the spleen of humanized mice and eliminated HIV-infected PBMCs. In addition, anti-HIV duoCAR-T cells effectively recognized and killed productive HIV-infected monocytes/macrophages in vitro, a cell type that contributes to the latent HIV reservoir. Last, we demonstrate efficient genetic modification of T cells from PWH on suppressive ART into anti-HIV duoCAR-T cells that subsequently killed autologous PBMCs superinfected with HIV.

**Conclusion:** The observed early-memory phenotype of anti-HIV duoCAR-T cells combined with markers of T cell activation and effector function suggest that our 8-day CAR-T cell manufacturing process generates a product that is primed to attack cells with active HIV infection and may provide a durable therapeutic response over time in PWH. These studies support translation of anti-HIV duoCAR-T cell therapy to PWH in our presently open phase I/II clinical trial (NCT04648046).

### 400 bNAB ESCAPE HIV-ENV VARIANTS IN ORALLY SHIV-INFECTED, ART-TREATED INFANT MACAQUES

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**Background:** Each year >150,000 children are newly infected with HIV, majority during breastfeeding. Early life infection forces adherence to life-long ART. Development of autologous virus neutralizing antibodies or broadly neutralizing antibody (bNAb) have been shown to limit HIV-1 clinical rebound after analytical treatment interruption (ATI). However, viral variants that escape neutralization response limit the therapeutic potential of these strategies. Our objective is to map the neutralization escape mutations in orally SHIV-infected infant rhesus macaques (RMs) following ATI with and without therapeutic immunization.

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

**Results:** While equivalent numbers of RMs from ART and ART+vaccine group developed autologous virus neutralizing antibodies (ART:5/10; ART+vaccine:5/9), the median titer was — five-fold higher in the vaccine group (ID50=557) vs. the ART group (ID50=124) RMs, who developed response (~ five-fold higher in the vaccine group (p = 0.16). Individual assessment of HIV-Env sequences from animals who rebounded, post-ATI between ART (18 days) or ART+vaccine group (14 days) (p = 0.01). Interestingly, RMs showed a glycine gain at position 332, which is associated with V3 bNAb resistance, even before ART start (Fig 1). No specific differences in
401 BISPECIFIC ANTIBODIES PROMOTE NK CELL-MEDIATED ELIMINATION OF THE HIV-1 RESERVOIR

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Background: The persistence of long-lived HIV-infected cells comprising the latent reservoir is the main barrier to an effective cure. One strategy for clearing the latent reservoir, known as “shock and kill,” involves selective reactivation of HIV gene expression through treatment with a latency reversing agent (LRA) followed by immune-mediated elimination of HIV-infected cells. To ensure effective elimination of HIV-infected cells in the context of “shock and kill,” novel immunotherapies must be developed to enhance HIV-specific cell-mediated cytolytic activity.

Methods: Here, we report single-chain diabody (scDb) constructs that target the HIV envelope protein (Env) and the human type III CCR5 receptor (CD16a). Two HIV-1-specific scDbs were designed based on PG9 and 3BNC117, broadly neutralizing antibodies specific for the V1/V2 and CD4 binding site regions of Env, respectively. We evaluated the ability of the scDbs to promote HIV-specific natural killer (NK) cell activation and NK cell-mediated cytolytic killing of infected cells in vitro. Using the intact proviral DNA assay and viral outgrowth assays, we quantified changes in the frequencies of infected CD4+ T cells following treatment with scDbs. The ability of scDbs to efficiently kill Env-expressing cell lines and HIV-infected primary cells was evaluated by flow cytometry and droplet digital PCR (ddPCR). The cytotoxic capacity was assessed using HIV envelope-expressing cell lines and HIV-infected autologous PBMCs or CD4+ T cells.

Results: Both scDbs promoted robust and HIV-specific NK cell activation and lysis of infected cells in vitro. These effects were not observed in an isotype control scDb targeting an irrelevant cancer epitope. Further, the Env-specific scDbs mediated ex vivo clearance of cells harboring intact proviruses (mean reduction 44%, range 20-67%, p < 0.0001). The observed clearance of reservoir cells was highly dependent on efficient latency reversal. Notably, we did not detect changes in cells harboring defective proviruses following ex vivo latency reversal and co-culture, suggesting that the scDbs are highly specific for cells expressing sufficient surface Env. Viral outgrowth assays revealed comparable scDb-mediated reductions in cells harboring inducible, replication-competent proviruses (mean reduction 40%, range 20-61%, p < 0.001).

Conclusion: Our study provides proof-of-concept evidence that the HIV-specific, NK cell-engaging scDbs described here are capable of mediating potent elimination of HIV reservoir cells. In combination with effective LRAs, the scDbs merit further preclinical evaluation as potential therapeutics for use in “shock and kill” HIV cure strategies.

402 DONOR-DERIVED ENV MUTATION SCANNING REVEALS aNAB AND bNAB VULNERABILITIES

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Background: Rapid viral evolution during untreated HIV-1 infection results in escape from autologous neutralizing antibodies (aNAbs). Escape often occurs within variable regions of the env gene, and at known epitopes of broadly neutralizing antibodies (bNAbs). Following ART initiation, viral evolution ceases, but aNAb activity persists against some viral variants in the latent reservoir. We hypothesized that identifying common escape mutations to aNAb will offer insight into the efficacy of passive and active immunization strategies.

Methods: We have previously characterized neutralizing activity of aNAbs against autologous envelope pseudo-typed viral particles from individuals suppressed on ART. Neutralization data were matched with the corresponding env sequence, and a genetic signatures analysis (Bricault et al., CHM 2019) was performed to identify putative determinants of aNAb resistance. Single amino acid sites of interest were altered using site-directed mutagenesis (SDM) in sensitive envs to adopt the resistant genotype. Changes in neutralizing activity of aNAbs and bNAbs against altered envelope pseudoviruses were investigated.

Results: Several amino acid sites were identified by bioinformatic analysis as having statistically significant association with aNAb resistance phenotype in a donor, 012. These single amino acid sites were altered by SDM in a sensitive env to reflect the resistant genotype. In this envelope sequence, a single alteration at the N332 glycan super-site—adding a glycan—significantly reduced the capacity of aNAb to neutralize the viral variant. Conversely, removal of the N332 glycan in a resistant envelope did not rescue neutralizing activity. Phenotypic changes were validated using the V3 glycan-directed bNAb 10-1074, that is highly specific for the presence of a glycan at N332. Moreover, several other alterations did not impact aNAb neutralization capacity.

Conclusion: These studies confirm the use of our experimental protocol to identify sites of aNAb and bNAb vulnerability within select envelope sequences. Only a single amino acid modification had the capacity to induce significant change in aNAb activity. This suggests that vulnerability to aNAb in latent reservoir viral variants could be the result of only a few instances of immune escape. Thus, immunization-based strategies for a cure may involve targeting a few key vulnerabilities in env.

403 HIV-RESISTANT CAR T CELLS BY CRISPR/Cas-MEDIATED CAR INTEGRATION INTO THE CCR5 LOCUS

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Background: The HIV reservoir is a major barrier to a cure. To augment T cell-mediated eradication of infected cells, we have engineered chimeric antigen receptor (CAR) T cells using a single-chain variable fragment (scFv) from the clinically potent broadly neutralizing antibody (bNAb) 10-1074. We used the CRISPR/Cas9 system to integrate the CAR expression cassette into either the AAVS1 locus (AAVS1-CAR) as a negative control or the CCR5 locus (CCR5-CAR) leading to concurrent CAR gene integration and CCR5 disruption, making the engineered CAR T cells resistant to HIV infection.

Methods: HIV-resistant CAR T cells were produced from healthy donor leukapheresis products and engineered by homology directed repair by delivering sgRNA/Cas9 ribonucleoprotein complexes by nucleofection and donor DNA templates by AAV6 viral vector transduction. CAR integration into T cells was evaluated by flow cytometry and droplet digital PCR (ddPCR). The cytotoxic capacity was assessed using HIV envelope-expressing cell lines and HIV-infected autologous PBMCs or CD4+ T cells.

Results: Engineered CCR5-CAR T cells showed complete CCR5 knock-out and protection from HIV infection. A mean of 20% (range 10-35%) of AAVS1-CAR and CCR5-CAR T cells expressed the CAR with precise CCR5 integration verified by ddPCR in CCR5-CAR. Both AAV51-CARs and CCR5-CARs showed no off-target effects towards autologous B cells and reporter cells not expressing HIV Env in 24-hour cytotoxicity assays. Specifically, we show that our AAV51-CAR and CCR5-CAR T cells lysed YU2-expressing Raji cells (81% and 77%, respectively) and RS-tropic NL4-3-infected autologous CD4+ T cells (67% and 63%, respectively) following 24-hours of co-culture in 2-4 biological replicates.
Finally, in a five-day co-culture with H828-infected CD8-depleted PBMCs, the AAVS1-CAR and CCR5-CAR T cells led to significant reductions (98.8 % and 93.3 % respectively, p < 0.05) of p24 levels in supernatants measured by ELISA.

**Conclusion:** In conclusion, we have developed HIV-specific CAR T cells with complete CCR5 disruption and protection from infection using the CRISPR/Cas9 system. Our cells showed no off-target effects and high cytotoxic capacity against HIV envelope-expressing cells. These data support the further development of gene edited cellular therapies to achieve an HIV cure.

### 404 Generating Broadly Neutralizing Antibody-Secreting CAR-T Cells Against HIV Infection

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**Background:** Treatment with HIV-specific CAR T cells and broadly neutralizing antibodies (bNabs) are both important approaches to potentially provide the sustained anti-HIV activity required to achieve ART-free HIV remission. We hypothesized that the anti-HIV activity of CAR-T cells could be further increased by enabling them to secrete bNabs. As a proof-of-concept, we generated a lentiviral vector (LV) encoding the BNC117 bNab (3BNC) which was co-transduced with a LV encoding an anti-HIV duoCAR construct targeting two distinct HIV gp120 epitopes with potent anti-HIV activity (Sci Transl Med. 2019; 11:aaw665) to generate 3BNC-secreting duoCAR-T cells.

**Methods:** CD3 T cells were purified from HIV-seronegative donors and transduced with 3BNC and/or HIV-duoCAR LV and evaluated for in vitro anti-HIV activity and 3BNC production. In vivo function was determined by intrasplenically co-injecting NSG mice with LV-transduced T cells and autologous PBMCs infected with an HIV infectious molecular clone expressing a fourth generation lentiviral vector harboring HIV-1 gag-pol. To assess if viral reservoirs were transcriptionally active (vRNA+), viral reservoirs were identified by single-cell RNA flow-cytometry (RNAflow-FISH) using probes targeting LTR-gag, gag or pol regions. To assess if viral reservoirs were transcriptionally active, viral reservoirs were identified by single-cell RNA flow-cytometry (RNAflow-FISH) using probes targeting LTR-gag, gag or pol regions.

**Results:** In vitro studies demonstrated that we could generate duoCAR-T cells capable of producing 3BNC that suppressed HIV infection. In the in vivo study, T cells transduced with the duoCAR and/or 3BNC LV (40 x 10^6 cells) were co-transduced intrasplenically into NSG mice with autologous PBMCs infected with HIV-Luc (20 x 10^6 cells). Prior to injection, ~50% of T cells expressed 3BNC or co-injected intrasplenically into NSG mice with autologous PBMCs infected with HIV-Luc (20 x 10^6 cells). Upon injection, ~50% of T cells expressed 3BNC or duoCAR only with single LV transduction. In the T cell transduced with both LVs (duo-LV), ~40%, 5% and 15% of them expressed 3BNC alone, duoCAR alone or both, respectively. After 3 weeks, CD4 T cells were preserved in the spleens of mice treated with 3BNC (35%), duoCAR (36%) and duo-LV (37%) transduced T cells, as compared to untreated mice (2%). In all treated mouse groups, HIV infection was suppressed by more than 90% as compared to untreated controls. Importantly, HIV levels in the duo-LV mice was 1.2 and 2.6 fold lower than 3BNC and HIV-duoCAR LV mice, respectively. Mice plasma 3BNC concentrations at 2 weeks after injection with 3BNC- or duo-LV-transduced T cells were 1.2 or 1.0 microgram/ml, respectively.

**Conclusion:** We successfully engineered HIV-CAR-T cells that secreted bNabs which exhibited superior in vivo anti-HIV function as compared to single LV-transduced T cells. This “2 in 1” approach provides a new strategy to generate more potent immunotherapeutics to contribute to a functional cure of HIV infection.

### 405 Circulating Immune Predictors of Intact HIV Reservoir Decay During Long-Term ART

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**Background:** The HIV reservoir is not stable during antiretroviral therapy (ART). Cells harboring intact genomes decay more rapidly than those with defective genomes, particularly during the first several years of therapy. The host factors associated with the rate of decay have not been characterized.**Methods:** We measured intact proviruses in PWH on ART using the intact proviral DNA assay (IPDA) in peripheral blood. We used Luminex bead-based multiplexed immunoassay system to measure 32 pro-inflammatory and regulatory cytokines in plasma. Based on our previous study focused on intact HIV genome kinetics, we fitted linear spline models with a knot at seven years and a random intercept and slope up to the knot. We estimated the influence of baseline cytokine levels and their trajectories on intact HIV kinetics in separate models.

**Results:** We studied 76 PWH on effective ART for a median of 10.4 (range 4.3 -15.6) years, providing a median of 3 (2 – 4) samples during the follow-up. Their median nadir CD4 was 180 (0 – 644) cells/µl and their baseline CD4 counts were 591 cells/µl (173-1600). Baseline galectin-9 was the most predictive marker of intact HIV kinetics: per each 10-fold decrease at baseline, there was a mean 45% greater reduction of intact HIV genomes per year (p = 0.0021; after adjustment for CD4 nadir, p = 0.0055). In contrast, lower baseline IFN-α, IL-17, and MIP-1α were predictive of intact HIV increases (Figure 1a). MIP-3α and IL-6 exhibited the strongest associations between longitudinal changes in cytokine level during ART and intact HIV kinetics. For each 10-fold increase of MIP-3α over time, we observed a concurrent 9.5% faster decay of intact HIV genomes (p=0.021), while for each 10-fold reduction of IL-6, intact genomes decreased 10% faster per year (p=0.043)(Figure 1b).

**Conclusion:** The extent of intact HIV decay was predicted by baseline galectin-9 levels, while MIP-3α and IL-6 correlated with intact HIV kinetics. Galectin-9 was the host factor most strongly associated with subsequent intact HIV decay, in alignment with its established roles in regulation of HIV expression and cytotoxic immunity.

Figure 1. Effects of baseline cytokine levels (a) and changes per year (b) on intact HIV reservoir kinetics.
immunologic signatures, at the cellular and transcriptional level, that correlate with augmented HIV-specific T cell responses post-vaccination in 30 participants with HIV who received an HIV DNA vaccine/IL-12 in the PENNIVAX clinical trial (NCT03606213).

Methods: Response to vaccination was defined as the fold change (FC) in the magnitude of HIV-specific T cell responses (averaged per HIV antigen) from pre-vaccination to 2 weeks post-boost (Week 14), as measured by IFNγ ELISPOT using vaccine-matched peptide pools (median FC 2.1 range 0.1-15.9). We characterized 473 innate and adaptive immune cell features using mass cytometry (cyTOF) and performed whole-blood RNA-sequencing. We then correlated cyTOF features and genes with ELISPOT FC to identify cell subsets and immune pathways associated with enhanced vaccine responses.

Results: Pre-vaccination, higher expression of genes involved in T cell homoeostasis (e.g., IL7, IL17R, adj. p=1e-04) and innate immune sensing (e.g., TLR9, IFNAR1, IL1R1; adj. p=8e-04) pathways were associated with a greater increase in HIV-specific T cell responses after vaccination (genes in Fig. 1A).

Individuals with a larger FC in their T cell response between baseline and Week 14 also demonstrated greater induction of genes in IL-2/STAT5-Signaling (adj. p=0.003) and hematopoiesis (adj. p=4e-09). By cyTOF, larger FC of ELISPOT responses post-vaccination were significantly (p=0.05) associated with a greater increase in the frequency of cytokine-skewed CD4+, CD8+, and γδ T cells expressing Granzymes A/B, Perforin, CX3CR1, and Tbet (Fig. 1B).

Conclusion: Our data highlight that individuals who develop a larger T cell response to a DNA HIV therapeutic vaccine have a baseline immune environment that promotes T cell survival and responsiveness to innate immune signaling, and that they respond to vaccination with more robust IL-2 signaling and hematopoiesis. We also demonstrate that therapeutic vaccination with DNA/IL-12 induces not only cytotoxic CD8+ T cells, but also cytotoxic CD4+ and γδ T cells. These data provide insight into how different host immune responses pre- and post-vaccination can impact vaccine immunogenicity and highlight that modulation of the pre-existing immune environment (e.g., with different adjuvants) may be critical to conditioning better vaccine responses. Immunologic features associated with a greater magnitude increase in T cell responses to therapeutic vaccination.

409 ANTIRETROVIRAL THERAPY REPAIRS CD4 T CELL DYSREGULATION IN PEOPLE LIVING WITH HIV
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Background: Recent studies show that the majority of the HIV reservoir is stabilized at the time of ART initiation. We hypothesized that ART in people...
with HIV (PWH) contributes to this stabilization by restoring CD4 T cell memory formation.

**Methods:** We used a 32-marker mass cytometry panel to examine CD4 T cell memory dynamics in PWH who are durably (~5 yrs) ART suppressed (PWHART, n=10) and PWH in the 1.5 years following ART initiation (n=10, ACTG5248). The panel included markers of activation (HLA-DR, CD38, CR3), activation/ exhaustion (PD-1), proliferation (Ki67), survival (Bcl-2) and long-lived memory (CD127). We paired these CD4 T cell studies with functional analysis of proliferation and T cell receptor (TCR) B cellotype sequencing.

**Results:** Using fixed effects models with indicators for participant ID, we found that frequencies of CD4 T cell memory subsets and expression of activation, exhaustion, cell cycling and long-liveness are remarkably stable in PWH-ART. In contrast, in the weeks after ART initiation, markers of activation and cell cycling decreased rapidly. Subsequently, slower but sustained increases in frequency of markers of T cell long liveness, CD127 and Bcl-2, were observed. Elastic net regression analysis was complementary, finding that CD127 Bcl-2 expression on both CD4 and CD8 central memory T cells increased post-ART initiation. TCR sequencing in PWHART found that dominant CD4 T cell clonotypes were not stable over time (17-19 months), however clonotypes that dominated (2-10%) at ART initiation were maintained for the first 18 months. To assess functionality of CD4 T cells post-ART, we measured CD4 T cell proliferation in response to pp65/IE1 CMV and mitogen stimulation. Proliferation was clearly detectable at ART initiation and decreased over time as viral load decreased. After viral suppression, proliferation began to recover. By contrast, we did not detect HIV Gag/Nef specific CD4 T cell proliferation over background.

**Conclusion:** In our study population (CD4 nadir 77-453), CD4 T cells were highly activated but retained proliferative capacity at ART initiation. ART resulted in rapid decreases in cell cycling and activation. Interestingly, restoration of CD4 T cell memory phenotypes was much slower, occurring over the months-years following virus suppression. CD4 T cell restoration likely supports maintenance of dominant CD4 T cell clonotypes at ART initiation. Overall, our data suggest that immune changes at ART initiation help stabilize the HIV reservoir in CD4 T cells.

### 410 REPROGRAMMING CMV-SPECIFIC CD8+ T CELLS TO TARGET HIV USING TCR GENE TRANSFER

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**Background:** Due to the chronic nature of HIV infection, cytotoxic CD8 T lymphocytes (CTLs) are limited in their capacity to provide sustained control of HIV infection in part due to exhaustion. In contrast, chronic cytomegalovirus (CMV) infection induces a unique CTL response termed memory inflation characterized by continued expansion following priming, maintained “effector-memory” phenotype, and sustained functionality. As T cell activation is crucial to enable lentivector transduction, we hypothesized that we could selectively transduce CMV-specific CTL with a lentivector expressing an HIV-specific TCR by using a novel immunotherapeutic, CMV-synTac, which selectively activates and expands CMV-specific CTL (JCI 2021;131:e141051). This would convert CMV-specific CTL into bispecific TCR capable of suppressing HIV infection while exhibiting the memory inflation phenotype.

**Methods:** After CMV-synTac treatment, CMV-specific CTL were selectively transduced with a lentivirus encoding a chimeric T cell receptor (TCR) containing a murine constant region fused to a human variable region specific for the HIV-1 Gag epitope, SL9. Use of the mouse constant region prevents mispairing of endogenous and lentivector-encoded alpha and beta TCR chains. TCR Expression was determined by tetramer staining and functional activity of the bispecific CTL was detected by ELSpot.

**Results:** CMV-specific CTL were selectively transduced with an SL9-specific TCR lentivector after activation with CMV-synTac, but not after anti-CD3/CD28 activation. Lentivector-encoded SL9-TCR function was demonstrated by markedly increased luciferase expression after coculturing SL9-TCR lentivector-transduced Jurkat T cells with an NFAT-regulated luciferase reporter with SL9-peptide but not irrelevant peptide-loaded T2 cells. Dual expression of the exogenous SL9-TCR and endogenous CMV-TCR in transduced donor CD8 T cells was detected by tetramer staining and flow cytometry. Lastly, functionality of both SL9- and CMV- TCRs were validated by interferon-gamma ELISpot assays after either SL9 or CMV peptide stimulation.

**Conclusion:** CMV-synTac enables antigen-specific transduction of CMV-specific CTL with a lentivector expressing an SL9-TCR which confers them with additional functional activity against HIV-infected cells. We anticipate that these bispecific CTLs should also express the memory inflation phenotype of the CMV-specific CTL enabling them to display sustained anti-HIV activity after infusion into PWH and thereby contribute to a functional cure.

### 411 NOVEL BISPECIFIC ENGAGER REDIRECTS CMV-SPECIFIC CTL TO ELIMINATE HIV-INFECTED CELLS

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**Background:** We developed a novel biologic (RediTac) to selectively activate and expand virus-specific memory CD8 T cells (CTLs), redirect them to eliminate HIV-infected cells, and thereby eliminate reactivated latent HIV-infected cells. RediTac employs a bispecific Fc-fusion protein scaffold to dimerize HLA-A2 pMHC linked to HIV bNAb scFvs. The pMHC presents a tethered viral peptide for selective binding and stimulation of cognate TCR on virus-specific CTLs, while the scFv redirects the CTL to eliminate HIV-infected cells. As a proof-of-concept, we constructed RediTac with pMHC presenting a CMV-derived peptide, NLY, which is associated with a well-described memory inflation phenotype. The NLY-pMHC was linked to an scFv derived from VRCo1, an HIV-1 Env-specific bNAb. This NLV-VRCo1 RediTac was tested to determine its ability to redirect CMV-specific CTLs to eliminate HIV-1 Env-expressing cells and suppress HIV-1 infection.

**Methods:** Donor PBMC treated with NLV-VRCo1 RediTac to expand NLV-specific memory CTLs were cocultured with either an Env-expressing 293T cell line or autologous CD4 T cells infected with an HIV infectious molecular clone expressing a luciferase reporter. NLV-VRCo1 RediTac or control treatments were administered and Env-expressing 293T cell killing or luciferase activity reduction was measured as a readout for anti-HIV function.

**Results:** Two weeks after NLV-VRCo1 RediTac treatment, NLV-specific CTLs expanded by >50-fold compared to untreated controls. Following a two-day coculture, these CTLs eliminated ~40% of Env-expressing 293T cells when treated with fresh NLV-VRCo1 RediTac. No elimination was observed when control non-cognate-VRCo1 RediTac or NLV-antiCD19 scFv RediTac was added instead. The addition of NLV-VRCo1 RediTac without NLV-specific CTLs suppressed HIV infection of donor CD4 T cells by ~35%, indicating neutralizing activity of the VRCo1 scFv domain. When NLV-VRCo1 RediTac was administered to HIV-infected donor CD4 T cells with syngeneic NLV-specific CTLs, the infection was suppressed by ~70% as compared to untreated cultures.

**Conclusion:** NLV-VRCo1 RediTac selectively expands CMV-specific memory CTLs and redirects their cytotoxic activity to eliminate HIV-infected cells, and in parallel, also functions as a bNAb to inhibit HIV-1 infection. These results support virus-specific CTL redirection as a novel approach to eliminate latent HIV-infected cells reactivated by LRAs. This approach could contribute to new strategies for a functional HIV-1 cure.

### 412 HIV-1 RESISTANCE PROFILES AGAINST bNAB IN INTACT VS DEFECTIVE GENOMES ARE DISTINCT

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**Background:** Broadly neutralizing antibodies (bNAbs) targeting HIV-1 Env are under study as a strategy in cure research. Viral env is genetically diverse, with resistance profiles relative to defectives.

**Results:** CMV-synTac enabled antigen-specific transduction of CMV-specific CTL with a lentivector expressing an SL9-TCR which confers them with
Methods: HIV-1 DNA genomes were sequenced from 1 virome and 11 viologically suppressed individuals on long-term ART via single genome amplification (HXEB 638-9632). Genomes were classified as intact or defective using HIVSeqR software. Resistance features against CD4 binding site-, V2-, and V3-targeting bNAbs examined include specific env residues, as well as hypervariable loop characteristics such as total length, number of N-linked glycosylation sites (PNGs), and electric charge.

Results: Of the 477 genomes obtained, 72 were intact, while full-length HIV-1 env was only present in 26% (106/405) of defective genomes. All 12 study donors displayed distinct resistance profiles against CD4abs, V2-, and V3-targeting bNAbs (Figure 1A), and all env in intact genomes were genetically unique compared to env in defective genomes within host. In the virome donor, 91% defective viruses lacked env, whereas the 20 intact genomes had two distinct bNAb resistance profiles, equally split, one with significantly more resistant residues, as well as longer V1+V2 loop lengths and a higher number of PNGs (p = 0.004) — all characteristics associated with higher V3 bNAb resistance (Bricault et al., 2019). In-depth analysis of the virologically suppressed donor with the most sequences revealed that resistance profiles in intact env were more homogeneous and displayed more sensitive profiles when compared to the defective envs (Figure 1B). Env in defective genomes had significantly longer V1+V2 hypervariable loops than intact env (p = 0.005) and were more negatively charged (p = 0.002). Two glycans, at positions 289 and 743, both associated with increased V3 bNAb resistance, were exclusively found in defective genomes.

Conclusion: Our results reveal that resistance profiles across all bNAb classes in env are diverse across hosts and distinct between intact and defective genomes within host, and highlight the importance of understanding intact-genomes resistance profiles when screening individuals for enrollment in bNAbs clinical trials.

Figure 1.

413 KIR+RA+CD8 T CELLS EXHIBIT ANTIGEN INDEPENDENCE AND CORRELATE WITH HIV RESERVOIR SIZE

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Background: CD8+ T cells from HIV+ individuals were sorted into distinct CD3+CD8+ subsets: TN (CD45RA+panKIR-CD27+), KIR+RA+ (CD45RA+panKIR+), TEMRA (CD45RA+ panKIR-CD27-), TCM (CD45RA-CD27), and TEM (CD45RA-CD27+). Cells were then infected with HIV and cocultured for 7 days with CD8+ T cells to assess for virus (Kag et al., 2015). The impact of IL-15 superagonist (N803) on KIR+RA+ was also assessed (n=5).

Methods: HIV-1 infection was only present in 26% (106/405) of intact genomes. All 12 study donors displayed distinct resistance profiles against CD4abs, V2-, and V3-targeting bNAbs (Figure 1A), and all env in intact genomes were genetically unique compared to env in defective genomes within host. In the virome donor, 91% defective viruses lacked env, whereas the 20 intact genomes had two distinct bNAb resistance profiles, equally split, one with significantly more resistant residues, as well as longer V1+V2 loop lengths and a higher number of PNGs (p = 0.004) — all characteristics associated with higher V3 bNAb resistance (Bricault et al., 2019). In-depth analysis of the virologically suppressed donor with the most sequences revealed that resistance profiles in intact env were more homogeneous and displayed more sensitive profiles when compared to the defective envs (Figure 1B). Env in defective genomes had a significantly longer V1+V2 hypervariable loops than intact env (p = 0.005) and were more negatively charged (p = 0.002). Two glycans, at positions 289 and 743, both associated with increased V3 bNAb resistance, were exclusively found in defective genomes.

Conclusion: Our results reveal that resistance profiles across all bNAb classes in env are diverse across hosts and distinct between intact and defective genomes within host, and highlight the importance of understanding intact-genomes resistance profiles when screening individuals for enrollment in bNAbs clinical trials.

Figure 1.

414 IMPACT OF SARS-CoV-2-MEDIATED CD4 T CELL ACTIVATION AND HIV DNA PERSISTENCE IN VIVO

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Background: Antigen-driven CD4++ T cell proliferation is a proposed mechanism of HIV-1 reservoir persistence. We previously reported that SARS-CoV-2 infection led to increased detectable low-level HIV-1 plasma RNA 6 months after COVID-19, but the impact of SARS-CoV-2-mediated T cell activation on expansion of HIV-1 reservoirs is not known. We sought to identify if SARS-CoV-2 infection leads to expansion of preferentially HIV-infected CD4++ T cells in people with HIV (PWH) on ART.

Methods: Five PWH with samples collected prior to and approximately two months after SARS-CoV-2 infection were identified. We performed a surface activation induced marker (AIM) assay using a CD4-optimized overlapping SARS-CoV-2 peptide pool to measure OX40/CD137 expression following peptide stimulation and sorted CD4++ T cells based on surface marker expression. ddPCR quantification of genomic HIV-1 DNA was performed on sorted subsets.

Results: We observed an increase in the frequency of SARS-CoV-2 AIM+ non-naive CD4++ T cells following COVID-19 in samples from 4 of 5 participants (mean AIM+ %: 0.13 pre- vs. 0.31 post). A large percentage of non-naive AIM+ CD4++ T cells expressed PD1 compared to total non-naive cells before (76% vs 36%) and after (65% vs 19%) COVID-19; PD1 expression was lower following SARS-CoV-2 in both AIM+ and AIM- CD4++ T cell subsets (although very few cells were AIM+ prior to COVID-19). HIV-1 DNA levels in non-naive AIM- CD4++ T cells prior to COVID-19 unexpectedly decreased following infection (mean 3,522 to 766 copies/106 cells). The numbers of AIM+ cells obtained by cell sorting were overall low (3.863 mean) and only one participant had detectable DNA in post-COVID AIM+ CD4++ T cells. However, a large majority of this participant’s post-COVID AIM+ cells harbored HIV-1 DNA (0.89 copies per cell) whereas HIV DNA in AIM- cells decreased from 8,387 to not detected following SARS-CoV-2 infection. No HIV-1 DNA was detected in the small number of AIM- cells obtained prior to COVID-19 in this participant.

Conclusion: COVID-19 in PWH led to a modest SARS-CoV-2-specific CD4++ cell response approximately two months following acute presentation. One participant may have preferentially expanded HIV-1-infected, SARS-CoV-2-specific CD4++ T cells following COVID-19 but studies involving larger numbers of participants and larger numbers of cells will be needed to fully understand the impact of SARS-CoV-2 on clonal expansion and HIV persistence.

415 UNRAVELING ONGOING INFLAMMATION AFTER EARLY ART BY IN-DEPTH ANALYSIS OF LYMPH NODES

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Background: Antigen-driven CD4++ T cell proliferation is a proposed mechanism of HIV-1 reservoir persistence. We previously reported that SARS-CoV-2 infection led to increased detectable low-level HIV-1 plasma RNA 6 months after COVID-19, but the impact of SARS-CoV-2-mediated T cell activation on expansion of HIV-1 reservoirs is not known. We sought to identify if SARS-CoV-2 infection leads to expansion of preferentially HIV-infected CD4++ T cells in people with HIV (PWH) on ART.

Methods: Five PWH with samples collected prior to and approximately two months after SARS-CoV-2 infection were identified. We performed a surface activation induced marker (AIM) assay using a CD4-optimized overlapping SARS-CoV-2 peptide pool to measure OX40/CD137 expression following peptide stimulation and sorted CD4++ T cells based on surface marker expression. ddPCR quantification of genomic HIV-1 DNA was performed on sorted subsets.

Results: We observed an increase in the frequency of SARS-CoV-2 AIM+ non-naive CD4++ T cells following COVID-19 in samples from 4 of 5 participants (mean AIM+ %: 0.13 pre- vs. 0.31 post). A large percentage of non-naive AIM+ CD4++ T cells expressed PD1 compared to total non-naive cells before (76% vs 36%) and after (65% vs 19%) COVID-19; PD1 expression was lower following SARS-CoV-2 in both AIM+ and AIM- CD4++ T cell subsets (although very few cells were AIM+ prior to COVID-19). HIV-1 DNA levels in non-naive AIM- CD4++ T cells prior to COVID-19 unexpectedly decreased following infection (mean 3,522 to 766 copies/106 cells). The numbers of AIM+ cells obtained by cell sorting were overall low (3.863 mean) and only one participant had detectable DNA in post-COVID AIM+ CD4++ T cells. However, a large majority of this participant’s post-COVID AIM+ cells harbored HIV-1 DNA (0.89 copies per cell) whereas HIV DNA in AIM- cells decreased from 8,387 to not detected following SARS-CoV-2 infection. No HIV-1 DNA was detected in the small number of AIM- cells obtained prior to COVID-19 in this participant.

Conclusion: COVID-19 in PWH led to a modest SARS-CoV-2-specific CD4++ cell response approximately two months following acute presentation. One participant may have preferentially expanded HIV-1-infected, SARS-CoV-2-specific CD4++ T cells following COVID-19 but studies involving larger numbers of participants and larger numbers of cells will be needed to fully understand the impact of SARS-CoV-2 on clonal expansion and HIV persistence.
Methods: Samples were selected from a multi-centric Belgian cohort study (ACS), in which blood and inguinal lymph nodes (LNs) were collected during acute HIV infection (T0), upon reaching an undetectable plasma viral load on ART (UD) and 1 year later (UD+1). We performed multiplex immunosassay (Mesoscale Discovery) on plasma of 25 participants at T0 and UD. A total of 15 T0 and 8 UD+1 formalin-fixed paraffin embedded inguinal LNs were analyzed by multiplex immune fluorescence (IF) to determine inflammatory changes and activation. In addition, HIV DNAscope was used to quantify the frequency of HIV DNA+ (vDNA) cells. To maximize the sensitivity of the assay, coverage of subtype-specific probe sets was predicted based on near-full-length sequences. In case < 20 ZZ pairs were predicted to bind, custom probe sets were used.

Results: Plasma levels of CXCL10 and CXCL11 at UD were significantly higher in ACS-participants compared to healthy controls (p = 0.018, p = 0.015). CXCL10 was detected in a highly focal pattern within LNs and was positively correlated with pSTAT1 expression (r = 0.614, p = 0.001). Both showed a non-statistically significant downward trend between T0 and UD+1 (p = 0.46, p = 0.09) and foci of CXCL10 did not significantly decrease at UD+1, with expression levels of pSTAT1 or CXCL10.

Conclusion: There were concerns of remaining increased systemic inflammation, despite intervention with very early ART. In-depth analysis of lymph nodes during and after acute HIV-infection, may provide insight into the drivers of this inflammation.

Methods: We obtained peripheral blood (PB) from 16 PWH under effective ART. Memory CD4 T-cells were FACs-sorted into 4 populations: PD-1-CTLA-4- (double negative, DN), PD-1+CTLA-4- (PD-1+), PD-1-CTLA-4+ (CTLA-4+) and PD-1+CTLA-4+ (double positive, DP). Using the full-length individual proviral sequencing assay, we identified genetically-intact and defective genomes from each subset (954 total genomes; 56 intact). We also identified proviruses with intact p24 open reading frames (ORFs), as p24 protein expression is used to identify replication-competent HIV provirus, and proviruses with an intact nef ORF due to the role of Nef in HIV persistence.

Results: We observed that DN and PD-1+ cells had a higher estimated intact infection frequency compared to CTLA-4+ and DP cells, with evidence for PD-1+>CTLA-4+ (p = 0.04), DN >CTLA-4+ (p = 0.01) and DN >DP cells (p = 0.03). However, the difference in intact infection frequency between DN and PD-1+ cells was variable across different participants (p = 0.009). When we investigated the infection frequency of proviruses with an intact p24 ORF, we observed the order of PD-1+>DN >DP >CTLA-4+ with evidence for PD-1+>DN, PD-1+>CTLA-4+ and PD-1+>DP (all p < 0.0001). These differences were also participant-dependent (all p < 0.001). We found that CTLA-4+ and DP cells had the lowest estimated intact nef infection frequency compared to DN and PD-1+, with evidence for DP-1+>CTLA-4+, DP-1+>DP and PD-1+>DN (all p < 0.0001), and DN >CTLA-4+ (p = 0.002). These differences all varied across participants (p = 0.02 for all).

Conclusion: PD-1+ cells contain HIV proviruses that are likely to have intact ORFs for genes such as p24 and nef in the PB, which may affect studies of HIV persistence. Conversely, we have found that CTLA-4 expression is a marker for HIV provirus that is more likely to be defective and contain low levels of these intact ORFs. However, all differences were highly variable between participants, and consideration of additional cellular markers will likely be needed to consistently identify cells harbouring potentially replication-competent, HIV.
In the intestinal tissue model (n=7) using ING and IL15. Means with SEM are represented and statistical comparisons were performed using the Wilcoxon test. *p<0.05.

**419 MONOCYTE-DERIVED MACROPHAGES CONTAIN LONG-LIVED LATENT HIV RESERVOIRS**

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**Background:** There is substantial evidence that HIV persists in myeloid cells (monocytes/macrophages) in blood and tissues in virologically suppressed people with HIV (vsPWH). However, despite the likelihood that myeloid cells contribute to the size of the HIV reservoir and will likely rebound during treatment interruption, little is known about this reservoir. Specifically, there are limited studies investigating whether HIV within myeloid cells can be reactivated to produce functional virus in vsPWH. Here we report the implementation of a novel human monocyte-derived macrophage quantitative viral outgrowth assay (MDM-QVOA) in a longitudinal cohort of vsPWH.

**Methods:** The cohort included 10 vsPWH. All subjects were male, had undetectable viral loads, median CD4 levels of 708 cells/µl (range 450-1086), on long-term suppressive ART (range 4-14yr), and had no reported viral blips. The MDM and CD4 replication-competent and DNA reservoirs were assessed via MDM or CD4-QVOA and qPCR for HIV gag. QVOA virus functionality was assessed via sequencing and subsequent infection of a CD4 T cell-line. T cell contamination was assessed by flow cytometry and TCRβ qPCR.

**Results:** 10/10 subjects had detectable HIV gag DNA in MDM (median(m)=1.47cp/1e6) at approximately 10-fold lower levels than their CD4 counterparts (m=15.4cp/1e6). MDM and CD4 had undetectable levels of HIV RNA (gag and tat/rev), suggesting that the cells were latent at time of sampling. 6/10 subjects were assessed longitudinally (150-1250 days) and had stable levels of HIV gag DNA. 5/10 subjects had detectable IUPM in the MDM-QVOA (m=0.44 infectious units per million [IUPM]) and 9/10 in the CD4-QVOA (m=1.6 IUPM). 3/5 subjects with detectable UPM in the MDM-QVOA were assessed longitudinally and had latent reservoirs that were consistently reactivated over several years. Additionally, higher HIV DNA levels in MDM stratified with viral outgrowth assay. (MDM-QVOA) in a longitudinal cohort of vsPWH.

**Conclusion:** The proposed definition of a viral reservoir in the context of eradication, is a cell type that allows the persistence of replication-competent HIV on a time scale of years in patients on optimal ART. The data presented here provide evidence that MDMs should be considered an HIV reservoir as they now meet the proposed definition.
Monocyte-derived macrophages from vsPWH have consistent levels of HIV DNA and reactivatable reservoirs overtime.

**420 SYSTEMIC INFLAMMATION, GUT DYSBIOSIS AND GUT MICROBIOME IMPACT HIV DYNAMICS**

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**Background:** HIV persists in the human body, including the gastrointestinal (GI) tract where the virus causes cellular damage, microbial translocation and inflammation. The GI tract also acts as a source of rebounding virus upon cessation of ART. We investigate HIV dynamics within host between tissue reservoirs and evaluate biomarkers, viral reservoir characteristics and microbiome composition associated with viral dynamics in 8 participants of the Last Gift cohort.

**Methods:** To infer HIV dynamics within each participant, Bayesian discrete trait analyses (DTA) were conducted using HIV DNA single genome full env sequencing data across 33 tissues (10 to 25 per participant) including 7 GI tissues. A panel of 44 cytokines were measured perimortem in blood (ELISA and LumineX assays). Microbiome was characterized in GI tissue (bacterial 16S-rDNA sequencing). Bayesian hierarchical models were used to interrogate the association between viral migration between tissues and (1) HIV reservoir characteristics (size measured by ddPCR, diversity and genetic distance), (2) microbiome composition and (3) soluble biomarkers. The models included predictors of the source and recipient tissues, whether the tissues are within the same biological system, and host- and tissue-level effects.

**Results:** For inflammatory markers, our DTA showed that viral migration within, from or toward GI tissues (Fig.1A) was consistently associated with higher levels of markers of inflammation and homeostasis (e.g. IL22, RANTES) and lower blood levels of ICAM1 and lower blood levels of CD62L, a regulator of cellular responses in inflammation (Fig.1B). For viral characteristics, our DTA showed strong associations between lower genetic distance between viral populations at anatomical sites and increased viral migration between those sites. Among GI tissues, viral diversity in source and recipient tissues was also positively associated with viral migration. Finally, there was a positive association between viral migration and the abundance of Fusobacteria (marker of gut dysbiosis) in the source tissue, and Actinobacteria in the recipient tissue. These associations between lower genetic distance between viral populations at anatomical sites and increased viral migration between those sites. Among GI tissues, viral diversity in source and recipient tissues was also positively associated with viral migration. Finally, there was a positive association between viral migration and the abundance of Fusobacteria (marker of gut dysbiosis) in the source tissue, and Actinobacteria in the recipient tissue. This study further supports the impact of HIV reservoir composition on viral dynamics. We showed that systemic inflammation and microbial gut environment also impacted HIV dynamics. Our approach provides a robust way to evaluate host and tissue level markers associated with directional HIV dispersal within host.

**Conclusion:** This study further supports the impact of HIV reservoir composition on viral dynamics. We showed that systemic inflammation and microbial gut environment also impacted HIV dynamics. Our approach provides a robust way to evaluate host and tissue level markers associated with directional HIV dispersal within host.

A. Global host HIV dynamics of FL HIV env sequences from 2 participants.
B. Posterior distribution of the estimates for three selected biomarkers

**(predictors) associated with viral movement (outcome) obtained from a Bayesian hierarchical model (BHM).**
**Conclusion:** Implementation of T-TRACE with HIV capture sequences allows efficient in-depth transcriptomic and surface phenotypic analysis of transcriptionally-active reservoir cells, along with inducible clonally-expanded reservoir cells prior to ex vivo stimulation. Application of the approach on specimens from ART-suppressed PWH revealed clones of HIV reservoir cells shared between gut and blood which differ markedly within their transcriptomes and surface phenotypes.

**422 HIV VIRION PROFILING SUGGEST THAT CXCR3+CD4+ T CELLS ARE THE MAIN HIV-PRODUCING CELLS**

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**Background:** The identification of markers of the HIV reservoirs represents a key step towards a HIV cure. In the present study, we postulated that the identification of host molecules incorporated into HIV virions may help to identify their cellular origin.

**Methods:** We therefore assessed the incorporated protein profiles of plasmatic HIV virions of untreated viremic HIV-infected individuals collected during acute (N=19), chronic (N=10) or AIDS phases (N=10) or at the time of viral rebound post-analytical treatment interruption (ATI; N=8). Briefly, HIV virions were captured using anti-gp120/41 coated beads and characterized using mass cytometry panels composed of 57 isotope-labelled mAbs.

**Results:** We showed that HIV virions collected during AIDS or post ATI harbored distinct incorporated protein profiles as compared to HIV virions from plasma collected during acute or chronic phases (P<0.05). Interestingly, a large proportions (70-85%) of virions collected during acute, chronic or post ATI harbored CXCR3, while other molecules expressed on activated CD4 T cells (ICOS, Lag-3 or CD40L) were less frequently detected (30-40%; P< 0.05). We therefore conducted a series of in vitro experiments to determine whether the incorporation of the aforementioned molecules may rely on passive and/or active mechanisms. We investigated the potential interactions between the intracellular tails (IC) of CXCR3, ICOS, Lag-3 or CD40L with immobilized recombinant p7 or p24 proteins using biosensor interferometry-based assay. The data demonstrated that p7 protein interacted with the icCXCR3, while no other interaction was detected between icICOS, icLag3 or icCD40L and p7. Notably no interaction was detected between p24 protein and any host molecules tested, demonstrating the specificity of p7/icCXCR3 interactions. To further determine the affinity of p7/icCXCR3 interactions, p7 was immobilized and exposed to various concentrations of icCXCR3 ranging from 0 to 450 nM. The data demonstrated that p7 protein interacted with icCXCR3 protein with a constant of dissociation (KD) value of 0.3 × 10−6 M (300nM) (±2.05−11).

**Conclusion:** Taken together, the developed experimental strategy indicate that CXCR3 expressing CD4+ T cells may serve as a major HIV cellular compartment for HIV production.

**423 GLYCOENGINEERING ENHANCES HIV 10-1074 bNAb-DEPENDENT NATURAL KILLER CELL CYTOTOXICITY**

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**Background:** Broadly neutralizing antibodies (bNAb) are being clinically tested to cure HIV. Such bNAb are recognized for their potent neutralizing activity. However, beyond neutralization, the constant domains (Fc) of antibodies can elicit several innate immune functions, such as antibody-dependent cellular cytotoxicity by natural killer (NK) cells (ADCC). Antibody Fc glycosylation impacts these functions; however, whether Fc glycans (fucose, galactose, and sialic acid) impact the ADCC of bNAb currently being tested to neutralize live virus (Fig. 1B-D). However, non-fucosylated glycoforms exhibited higher levels of ADCC compared to fucosylated glycoforms (Fig 1E; P< 0.013). Galactosylated glycoforms also exhibited higher ADCC than agalactosylated glycoforms within fucosylated and non-fucosylated glycoforms (P=0.017).

**Results:** Further, the G2 glycoform (non-fucosylated, galactosylated, non-sialylated) exhibited ~4-fold enhancement of ADCC compared to WT 10-1074 (from 17.46% cytotoxicity with WT 10-1074 to 68.02% cytotoxicity with the G2 glycoform; Fig. 1E). As expected, the ADCC activity correlated strongly with antibody binding to Fcγ3a receptor (P< 0.0001; not shown).

**Conclusion:** Removing fucose and adding galactose to 10-1074 can significantly enhance its ADCC activity, which may improve its overall efficiency. Further studies are warranted to examine the impact of bNAb glycosylation on other innate immune functions, such as antibody-dependent cellular phagocytosis and antibody-dependent complement deposition ex vivo and in vivo. Together, these findings highlight the importance of optimizing bNAb glycosylation to potentially eliminate or functionally cure HIV.

**424 SINGLE-CELL QUANTIFICATION OF HIV-1 AND LENTIVIRAL VECTOR IN GENE THERAPY STUDIES**

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**Background:** Lentiviral-based vector therapies, which include many CAR-T and gene-modified cell therapies, are at the forefront of HIV-1 cure research. However, sequence homology between these lentiviral vectors and HIV-1 and gene-modified cell therapies, are at the forefront of HIV-1 cure research. As a result, we developed a novel single-cell in droplet (scd)PCR method to co-quantify genomic HIV-1 DNA and lentiviral vector DNA (from CAR-T and gene-modified autologous stem cell transplant) with single-cell resolution. This approach provides a high-throughput platform to determine if gene-modified cells can become infected in vivo, a critical unknown question.
Methods: We adapted a multiplexed scdPCR method to co-quantify genomic HIV-1 DNA and the MNDU3 promoter, a common component of lentiviral gene delivery vectors. In addition, we screened over 30 existing HIV-1 DNA quantitative assays to determine the optimal method to quantify HIV DNA in cells from human studies.

Results: First, we show in the figure that commonly used lentiviral vectors directly cross-react with the HIV-1 LTR, LTR-Gag junction/Psi, and Env regions. Second, we observed that the intact proviral DNA assay (IPDA), co-targeting Psi and Env, quantified nearly 100% of lentiviral vector DNA. Third, of over 30 screened HIV-1 qPCR assays, we identified only one, targeting the downstream Gag region, that did not have significant cross-amplification with lentiviral vector DNA. Fourth, we successfully duplexed the HIV-1 and MNDU3 DNA assays for droplet-digital (dd)PCR with minimal cross-amplification (Figure) using both physiological and super-physiological levels of HIV-1 and vector DNA in bulk cell lysates from combinations of 293T cells transfected with: a) HIV-1 plasmid alone, b) Tat (lentiviral vector), c) HIV-1 and Tat, d) not transfected. Finally, we have successfully applied the duplexed ddPCR assay in our scdPCR platform for use with individually encapsulated transfected 293T cells. The multiplexed ddPCR assay is now being implemented on participant-derived cells from a multiple gene modification study in autologous SCT (AMCO97) and a dUCAR-T cell study in people with HIV.

Conclusion: The multiplexed scdPCR assay can reliably quantify both HIV-1 DNA and lentivector vector DNA in both bulk cell lysates and in individual, encapsulated cells. This method also allows in-depth characterization of residual HIV-1 burden and the potential for infected, transduced cells in vivo/ex vivo across a range of gene modification studies.

Example of Cross Reactivity of Traditional LTR-Gag HIV-1 Region (Top) and Duplexing MNDU3 Viral Promoter and HIV-1 Gag Region (Bottom)

TRANSLATIONALLY ACTIVE HIV RESERVOIR

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Background: Despite the success of combination antiretroviral therapies (cART) to suppress HIV replication, HIV persists in a long-lived reservoir that can give rise to viral rebound upon cART cessation. The active reservoir consists of infected cells that continue to transcribe HIV and produce viral protein even in the presence of cART. These transcriptionally and translationally competent HIV-infected cells likely contribute to chronic immune activation in cART-suppressed, HIV-infected individuals and are implicated in rebound viremia upon cART cessation. Methodologies to quantify the active reservoir are needed.

Methods: We developed an automated immunocytochemistry (ICC) assay coupled with computational image analysis to detect and quantify intracellular Gag capsid protein (CA). For this purpose, we cytophinned fixed cells onto microscopy slides and used an automated stainer with antibodies against CA. Stained slides were digitized with a slide scanner, after which nuclear staining was used to enumerate total cells and chromogenic signal to quantify the percentage of CA-positive cells. We used qPCR, digital ELISA and flow cytometry to quantify plasma viral loads (pVL) and CA to validate CA-ICC with established assays.

Results: We determined the sensitivity of the CA-ICC assay using the Moi311B cell line spiked into uninfected Jurkat cells in limiting dilutions and assayed the specificity of the staining with peripheral blood mononuclear cells (PBMCs) from HIV-seronegative donors or after in vitro infection with an HIV laboratory strain. We then evaluated the application of this assay with mouse and non-human primate animal models to detect HIV-1 p24- or SIV p27-containing cells in PBMCs, respectively. The frequency of intracellular CA positive cells from both animal models’ PBMCs corresponded with the pVL and cell-associated CA. We also quantified CA-positive cells in PBMCs obtained from donors living with HIV on cART and found specimens in resting condition with detectable CA-positive cells above the background determined by cells from HIV-seronegative donors. This CA-ICC method allowed us to quantify the activity of small molecule targeted activators of cell kill (TACK) in eliminating CA-expressing cells.

Conclusion: We developed and validated an ICC assay to investigate the active HIV-1 reservoir. Efforts are ongoing to multiplex markers to further characterize the active HIV-1 reservoir and provide a benchmark towards a functional HIV-1 cure.

MULTI-SEROYPE NANOBODY-ENGINEERED AAV VECTORS FOR CD4 TARGETED GENE THERAPY

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Background: HIV cure approaches involving in vivo gene therapy have the potential to target prominent tissue-resident HIV reservoirs. Virus-based vectors are most common in clinical studies, with Adeno-associated Virus (AAV) being the vector of choice. Effectiveness in in vivo gene therapy largely correlates with high vector doses, but must be balanced to avert unwanted effects including immune responses and transgene activity in non-target cells. Modulating receptor binding specificities of therapeutic vectors increases on-target effectiveness while reducing off-target effects. So far, no robust viral gene therapy vector specific for CD4-positive HIV target cells has been described. We here show that coupling of AAV capsid surfaces with CD4-specific nanobodies mediates retargeting of AAVs to CD4-positive cells. These CD4-AAVs could provide an invaluable vector for anti-HIV therapeutic approaches.

Methods: We used structure-based rational design to genetically fuse CD4-specific nanobodies into AAV capsid proteins VP1 and VP2. We examined optimal nanobody/insertion-site combination for targeted reporter transgene delivery in vitro and in vivo. We applied our engineering platform to multiple AAV serotypes, proving system versatility. Electron microscopy (EM) and biochemical analyses were used to investigate particle characteristics.

Results: Functional competition assays with CD4-AAV vectors revealed highly specific transduction of CD4-positive cells in vitro. Isolated human primary CD4-positive T cells from multiple donors are effectively transduced compared to wild-type AAV serotypes. CD4-positive cells were transduced with 5-fold higher specificity compared to CD4-negative cells in vitro. CD4-specific transduction was also observed in humanized mice after IV delivery. EM and biochemical characterization of CD4-AAV particles revealed overall beneficial features: regular morphology, increased capsid stability, nanobody presentation on the capsid outer shell, exceeding titers and unaffected genome packaging compared to wild-type AAVs.

Conclusion: We provide proof-of-concept for a novel AAV-based vector platform with high specificity towards CD4-positive cells. These nanobody-engineered AAVs further preserve all beneficial AAV vector characteristic. Adaptation to other serotypes and exchange of nanobodies is straightforward.

AN IMMUNOCYTOCHEMISTRY METHOD TO INVESTIGATE THE TRANSLATIONALLY ACTIVE HIV RESERVOIR

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Background: The active HIV-1 reservoir and provide a benchmark towards a functional HIV-1 cure. Efforts are ongoing to multiplex markers to further characterize the active HIV-1 reservoir and provide a benchmark towards a functional HIV-1 cure.
427 DUAL ANTIRETROVIRAL-LOADED NANOPARTICLES FOR LONG-ACTING SUPPRESSIVE HIV THERAPY

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Background: Viral persistence in secondary lymphoid tissues (SLTs) of patients on cART has been linked to low SLT penetration of antiretrovirals (ARVs). Furthermore, suboptimal adherence to ARVs can lead to drug failure and rapid viral rebound from these SLTs. Therapeutic strategies that sustain ARV concentrations in SLTs are essential. Hence, we designed membrane-wrapped poly-lactic acid nanoparticles expressing the CD169 ligand GM3 (GM3+ NPs) and co-incorporating Filgrastim (RPV) and Cabotegravir (CAB) to selectively target CD169+ macrophages in SLTs. We hypothesized that GM3+ NP retention within CD169+ CD81+ non-degradable compartments (NPCCs) in macrophages will lead to establishment of myeloid cell-associated ARV depots for sustained viral suppression in SLTs.

Methods: GM3-NPs were formulated by one-step nanoprecipitation of lipids, poly-lactic acid, RPV and CAB. Intracellular ARV retention in monocyte-derived macrophages (MDMs) was quantified by high performance liquid chromatography (HPLC). Trafficking of NPs in MDMs was determined by quantifying co-localization of NPs with CD81+ NPCCs via confocal microscopy. Antiviral effect of NPs was assessed in HIV-1 infected macrophages (MDMs) using qPCR, measuring the levels of HLA-DR, CD38, PD-1, TIGIT, and intracellular ARV concentrations to determine the impact of this strategy on HIV blood and tissue latent reservoirs, residual viremia, immune activation, and inflammation.

Results: There was no significant difference in total HIV DNA measured in plasma and in tissue. Gene expression (HLA-DR, CD38) was decreased in treated compared to placebo animals (C11, C12) at day 21 post-injection (POI) in a single-dose mouse model, whereas no differences were seen in the control group. These results suggest that GM3+ NPs are an attractive long-acting delivery platform with the potential to enhance ARV pharmacokinetics and facilitate sustained inhibition of HIV-1 replication by establishing drug depots in CD169+ macrophages in SLTs.

Conclusion: Temporal HPLC analysis of MDM lysates revealed that GM3+ NPs maintained high intracellular ARV concentration for 28 days, which correlated with persistent localization of GM3+ NPs in CD81+ NPCCs. GM3+ NPs sustained antiviral effect in MDMs, with robust viral suppression for 35 days post NP addition. Furthermore, GM3+ NPs co-localized with CD169+ cells and were retained in SLTs for 21 days unlike GM3- NPs. Importantly, a single dose of ARV-GM3+ NPs suppressed viremia in HIV-infected BLT mice for 21 days to levels observed with daily NP-free RPV/CAB.

428 REDUCTION IN HIV RESERVOIR MARKERS WITH GAG/POL/IL-12 DNA THERAPEUTIC VACCINATION

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Background: HIV is a chronic viral infection characterized by viral persistence in secondary lymphoid tissues (SLTs) of patients on cART, which can lead to drug failure and rapid viral rebound from these SLTs. Therapeutic strategies that sustain ARV concentrations in SLTs are essential. Hence, we designed membrane-wrapped poly-lactic acid nanoparticles expressing the CD169 ligand GM3 (GM3+ NPs) co-incorporating Filgrastim (RPV) and Cabotegravir (CAB) to selectively target CD169+ macrophages in SLTs. We hypothesized that GM3+ NP retention within CD169+ CD81+ non-degradable compartments (NPCCs) in macrophages will lead to establishment of myeloid cell-associated ARV depots for sustained viral suppression in SLTs.

Methods: GM3-NPs were formulated by one-step nanoprecipitation of lipids, poly-lactic acid, RPV and CAB. Intracellular ARV retention in monocyte-derived macrophages (MDMs) was quantified by high performance liquid chromatography (HPLC). Trafficking of NPs in MDMs was determined by quantifying co-localization of NPs with CD81+ NPCCs via confocal microscopy. Antiviral effect of NPs was assessed in HIV-1 infected macrophages (MDMs) using qPCR, measuring the levels of HLA-DR, CD38, PD-1, TIGIT, and intracellular ARV concentrations to determine the impact of this strategy on HIV blood and tissue latent reservoirs, residual viremia, immune activation, and inflammation.

Results: There was no significant difference in total HIV DNA measured in plasma and in tissue. Gene expression (HLA-DR, CD38) was decreased in treated compared to placebo animals (C11, C12) at day 21 post-injection (POI) in a single-dose mouse model, whereas no differences were seen in the control group. These results suggest that GM3+ NPs are an attractive long-acting delivery platform with the potential to enhance ARV pharmacokinetics and facilitate sustained inhibition of HIV-1 replication by establishing drug depots in CD169+ macrophages in SLTs.

Conclusion: Temporal HPLC analysis of MDM lysates revealed that GM3+ NPs maintained high intracellular ARV concentration for 28 days, which correlated with persistent localization of GM3+ NPs in CD81+ NPCCs. GM3+ NPs sustained antiviral effect in MDMs, with robust viral suppression for 35 days post NP addition. Furthermore, GM3+ NPs co-localized with CD169+ cells and were retained in SLTs for 21 days unlike GM3- NPs. Importantly, a single dose of ARV-GM3+ NPs suppressed viremia in HIV-infected BLT mice for 21 days to levels observed with daily NP-free RPV/CAB.

429 DOUBLING DOLUTEGRAVIR DOSAGE REDUCES HIV PERSISTENCE MARKERS IN ART-TREATED ADULTS

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Background: Whether ongoing viral replication occurs in people living with HIV (PLWH) despite antiretroviral therapy (ART) and leads to low-level residual viremia is still debated. Here we report on a study, in which we intensified the ART regimen by doubling dolutingravir (DTG) dosage. We investigated the impact of this strategy on HIV blood and tissue latent reservoirs, residual viremia, immune activation, and inflammation.

Methods: Twenty HIV-infected adults, who received a triple therapy consisting of DTG 50mg/ABC/3TC and were fully suppressed for at least 2 years, were enrolled in a phase 3 randomized clinical trial. Half of them received an additional 50 mg of DTG as treatment intensification. Peripheral blood mononuclear cells (PBMCs), plasma and rectal biopsies were collected at different time points over 3 months. We quantified total HIV DNA, intact HIV RNA (IPDA), cell-associated unspliced (US) HIV RNA in PBMCs and in tissue. Single copy assay was performed to determine ultrasensitive plasma viral load.

Results: Twenty HIV-infected adults, who received a triple therapy consisting of DTG 50mg/ABC/3TC and were fully suppressed for at least 2 years, were enrolled in a phase 3 randomized clinical trial. Half of them received an additional 50 mg of DTG as treatment intensification. Peripheral blood mononuclear cells (PBMCs), plasma and rectal biopsies were collected at different time points over 3 months. We quantified total HIV DNA, intact HIV RNA (IPDA), cell-associated unspliced (US) HIV RNA in PBMCs and in tissue. Single copy assay was performed to determine ultrasensitive plasma viral load. Expression of immune activation (HLA-DR, CD38) and exhaustion (PD-1, TIGIT, LAG-3) markers on CD4+ and CD8+ T cells was evaluated. Inflammation was assessed by measuring the levels of several plasma biomarkers including sCD14, IL-1RA, IL-6, IP-10, uCSRP, IFN gamma, and TNF alpha. Concentration of dolutingravir was measured in plasma and in tissue.

Results: There was no significant difference in total HIV DNA in PBMCs and in tissue between day 0 and day 84 in both groups. However, we observed a significant decrease in US HIV RNA in PBMCs (p=0.020) and in ultrasensitive plasma viral load (p=0.016) between day 0 to day 84 in the intensified group, whereas no such differences were observed in the control group. These results...
suggest ongoing viral replication prior to intensification, even though we could not detect differences in immune activation or inflammation between the groups. These results should be confirmed in tissue where it would be even more relevant.

**Conclusion:** We observed a decrease in US RNA and unsuppressive plasma viral load following DTG intensification, suggesting ongoing viral replication in some participants. However, it had no measurable impact on chronic inflammation or immune activation. If confirmed in larger clinical trials, these results could have an impact on the clinical management of PLWH.

**430 RANDOMIZED CONTROLLED TRIAL OF VRC01 MONOCLONAL ANTIBODY DURING ACUTE HIV INFECTION**

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**Background:** The VRC01 broadly neutralizing monoclonal antibody (mAb), targeting the CD4 binding site of HIV-1, has been shown to decrease viremia and prevent infection with neutralization-sensitive strains. We investigated the impact of a single intravenous VRC01 infusion in acute HIV-1 infection in individuals who received antiretroviral therapy (ART) simultaneously or one week later.

**Methods:** RV398 (NCT02991420) was a randomized placebo-controlled trial of 24 adults enrolled with acute HIV-1 infection in Thailand, Kenya, Uganda, and Tanzania. Eight participants were randomized to each of the three arms: 1) placebo infusion + immediate ART, 2) VRC01 40mg/kg + immediate ART, or 3) VRC01 40mg/kg + subsequent ART initiated on day 7. Infusions in arms 1 and 2 were blinded; study duration was 24 weeks. Primary objectives were to assess mAb safety and the change in plasma viremia through day 7.

**Results:** Enrollment completed in September 2020 with 15 (63%) participants in Thailand and 9 (37%) in East Africa. Mean age (SD) was 23.4 (3.6) years; 14 participants (58%) were cis-men, 7 (29%) cis-women, and 3 (13%) transgender in Thailand and 9 (37%) in East Africa. Twelve (50%) were recruited in Fiebig stages I-II, 6 (25%) in III, and 2 (8%) in IV-V. There was one grade 3 mAb-related transient AST elevation and no mAb-related serious adverse events (AEs). Solicited AEs were mild or moderate. We observed a significantly greater viral load reduction by day 7 and prevent infection with neutralization-sensitive strains. We investigated the impact of a single intravenous VRC01 infusion in acute HIV-1 infection in individuals who received antiretroviral therapy (ART) simultaneously or one week later.

**Conclusions:** Initial results demonstrate the safety of VRC01 with ART in acute HIV infection and the feasibility of studying mAb interventions during AH1 across diverse subtypes and geographies. Further studies, including viral isolate mAb sensitivity, are ongoing to identify the determinants of the limited impact of VRC01 on acute HIV viremia.

Figure 1: Plasma Viremia Through Day 28

**431 BEATZ PRIMARY TRIAL OUTCOMES: PEG-IFN-a2b + 3BCN117 & 10-1074 IN CHRONIC HIV INFECTION**

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**Background:** BEATZ (NCT03588715) is an open-label 50-week study in ART-suppressed PLWH undergoing ART substitution with 26 weeks of immunotherapy (IMM-Tx) [pegylated interferon alfa-2b (peg-IFN-a2b) and broadly neutralizing antibodies (bNAbs)] 3BCN117 and 10-1074, followed by an IMM-Tx free ATI up to 24 weeks. Primary results at week 28 are presented.

**Methods:** Participants had baseline HIV bNAbs-sensitive virus on PBMC using the Phenosense mAb Assay (Labcorp-Monogram Bio.) (IC50 < 2.0 µg/mL) (3BCN117) and < 1.5 µg/mL (10-1074). Participants received A) 29 weekly doses of peg-IFN-a2b (1.5µg/kg) (Step 2) = 4 wks on ART and Step 3 = 26 wks off ART), and B) seven IV infusions of the bNAbs combination (30 mg/kg) of each at weeks 0, 2, 4, 8, 12, 16, 20 of Step 3. Step 4 (ATI: IMM-Tx cessation) was initiated after Step 3: Step 5 (ART re-start) triggered by 6 consecutive weekly measurements HIV VL > 1,000 c/ml. We measured reservoir size (IPDA) during Step 3, and bNAbs PK during Step 3 and 4. The primary end-point was not meeting ART re-treatment criteria in more than 10% (one sided 5% alpha level) at week 38. bNAbs sensitivity was evaluated upon viremia (plasma) and after re-starting ART when the HIV VL was < 50 c/ml (PBMC).

**Results:** We enrolled 14 participants (12 males, 8 African American) (median CD4 count = 618 c/mm3 (IQR 739-1079); nadir CD4 > 200 c/mm3). Two participants voluntarily discontinued the study due to bNAbs infusion-related chills. IMM-Tx regimen was safe and well tolerated and maintained ART-free HIV suppression (< 20 c/ml) for 26 weeks in 10/12 (83.3%) of participants. Primary endpoint: 4 of 14 participants (28.6%) did not meet ART re-treatment criteria at 12 weeks of IMM-Tx cessation, and 2 (14.3%) maintained < 50c/ml for > 50 weeks. This rate was greater than observed in non-intervention ATI historical controls (p=0.05). Variants resistant to both bNAbs emerged in two participants (14.3%) during IMM-Tx (Figure). BNAb escape was detected during viral rebound in step 4 in 6/10 (60%) after IMM-Tx stopped. Resistance to BNAbs was detected after ART re-suppression in 5/13 persons (38.4%). No change from baseline in IPDA was noted at end of IMM-Tx (26 wks of Step 3).

**Conclusion:** BNAbs and peg-IFN-a2b maintained 80% viral suppression for 26 weeks in the absence of ART, and results in subsequent improved viral control without effects on HIV reservoir. Selection of BNAbs escape was observed in 75% of participants during IMM-Tx cessation.

**432 LONG-TERM PROTECTION OF CD4+ T CELLS AGAINST HIV BY ONE-YEAR TREATMENT WITH PONATINIB**

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**Background:** Tyrosine kinase inhibitors (TKIs) interfere with the formation and replenishment of HIV reservoir by preserving SAMHD1 antiviral activity in
CD4 cells and impeding homeostatic proliferation. We evaluated the persistence of TKI-mediated protective activity against HIV in CD4 from individuals who received one-year treatment with ponatinib against chronic myeloid leukemia (CML).

**Methods:** Nine participants of Phase II clinical trial NCT04043676 were recruited. They achieved deep molecular response against CML after 14 (IQR 5.3-13.5) years of treatment with imatinib before interruption and then received one-year consolidation treatment with ponatinib 15 mg/d. Blood samples were taken before starting ponatinib (t=12 months), after one year of treatment (t=3, and 6 months after interruption). PBMCs were activated with PHA/IL-2 for 48h and then infected with NL4-3_v12 for 72h. HIV p24, SAMHD1 phosphorylation at T592 (pSAMHD1), and distribution of CD4 memory subpopulations were analyzed by flow cytometry.

**Results:** 1) 5 participants (55.5%) did not relapse from CML 12 months after ponatinib interruption (Non-relapsed), while 4 participants (44.4%) relapsed after 5.5 months (IQR 4.25-6.75) of ponatinib interruption (Relapsed). 2) CD4 were susceptible to HIV infection in all participants while they were treated with imatinib, but one-year treatment with ponatinib reduced 8.8-fold HIV infection in these cells (Figure). This protection was maintained after 12 months of treatment-free remission (TFR) in Non-relapsed (p=0.0039), in correlation with interference of pSAMHD1. 3) All CD4 memory subpopulations regained susceptibility to HIV infection after CML relapse and imatinib reintroduction. Therefore, p24 production was increased 6.1-(p=0.0159), 8.6-(p=0.0159), and 5.0-fold (p=0.0317) in CD4 naïve, TCM, and TEMRA cells of Relapsed, while CD4 of Non-relapsed were protected against HIV infection 12 months after ponatinib interruption. Increased p24 synthesis correlated with increased pSAMHD1 in CD4; pSAMHD1 levels were not significantly modified in CD8.

**Conclusion:** One-year treatment with ponatinib preserved SAMHD1 antiviral activity in CD4 by a potent sustained cytostatic effect, impeding HIV infection. The antiviral protection was maintained for 12 months during TFR in correlation with sustained antileukemic response. Transient use of TKIs may be applied to advance towards an HIV cure.

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**A PLACEBO-CONTROLLED RANDOMIZED TRIAL OF THE HTI IMMUNOGENIC VACCINE AND VESATOLIMOD**

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**Background:** To date, four persons living with HIV infection (PLWH) have achieved HIV cure through CCR5∆32 homozygous allogeneic stem cell transplantation (SCT). Post-SCT events leading to remission in this setting are uncertain, and no detailed analyses of HIV reactivation have been reported. Here we conducted virologic and immunologic analyses of HIV reactivation after CCR5∆32/∆32 SCT.

**Methods:** The HEME-17 protocol evaluates HIV-1 persistence in PLWH pre and post allogeneic SCT utilizing CCR5∆32/∆32 donors. Plasma and peripheral blood mononuclear cells (PBMCs) samples were analyzed by single-copy quantification of CCR5/CCR5∆32 (digital PCR, dPCR) and of HIV gag DNA and RNA (HMMIC-gag), and by single-genome sequencing (SGS) of HIV env. Plasma antibodies to HIV Gag, Env, and Nef were quantified by a luciferase immunoprecipitation system.

**Results:** A 67 yo male PLWH with 100% R5-tropic HIV undergoing ART with HIV RNA < 50 copies/ml for 14 years developed acute myeloid leukemia. The patient underwent a reduced intensity allogeneic SCT with a 10/10 CCR5∆32/∆32 matched unrelated donor. By day 100, 100% engraftment was achieved. HIV was not detected in plasma (<0.3 copies HIV-1 RNA/ml) or in PBMCs (<0.3 copy HIV RNA or DNA/million) at 3, 9, 12, and 15 months post SCT; no WCCR5 was detected in the PBMC at 1 year by dPCR (Fig 1). Antiretroviral treatment interruption (ATI) was initiated 15 months post-SCT. At ATI, weekly HIV-1 qPCR demonstrated undetectable HIV-1, (<20 copies/ml), until 8 weeks, when the plasma VL was detected at 780 copies/ml. A repeat VL, one week later, demonstrated a drop in the viral load to 300 copies/ml, when ART was re-initiated. Five (7) plasma HIV env sequences obtained at this time were genetically diverse and all had predicted R5 tropism. At viral rebound, HIV cell-associated DNA/RNA (< 0.14 copies/million) and wt CCR5 (< 20 copies/million) assays were used to evaluate immunogenicity, VES pharmacodynamic (PD) biomarkers and changes in viral reservoir, respectively.

**Results:** 50 participants were enrolled and 47 entered the ATI (CCMMA+vES [n=30] or placebo [n=17]). The intervention was well-tolerated with 1 unrelated SAE. Currently available immune data from 31/47 (66%) participants demonstrated strong immunogenicity. The total peak HTI-specific T cell response increased from baseline in the active arm, with median (range) increase of 2474 spot-forming cells (SFC)/10^6 PBMC (635 to 9300) vs. 610 SFC/10^6 PBMC (50 to 3645) in the placebo arm, respectively (p=0.001). Compared to AELIX-002, where HTI vaccines were given alone as DDDMM followed by CCM, peak total HTI-specific responses in AELIX-003 were significantly higher (p=0.027). At time of ATI start, a median (range) of 52% (13% to 100%) of the total anti-HIV 1 T-cell response was HTI-specific in the active arm vs 19% (0% to 100%) in the placebo arm (p=0.01). VES increased the production of antiviral cytokines (IFN-a, IL1RA) and chemokines (ITAC) 24h post-dosing. Decay in total and intact HIV proviral DNA from baseline to ATI was similar between arms. Ten out of 30 (33%) participants in the active arm remained off ART for 24 weeks compared to 24% (4/17) in the placebo arm.

**Conclusion:** In early-treated PWH, HTI vaccines and VES were safe and induced stronger HTI responses than the more complex AELIX-002 vaccine regimen. Mechanism of action and viral outcome correlate analyses are ongoing.

**Figure 1 AELIX-003 study design**

**434 HIV-1 VIRAL RESERVOIR REACTIVATION AFTER CCR5 DELTA 32/32 STEM CELL TRANSPLANTATION**

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**Background:** To date, four persons living with HIV infection (PLWH) have achieved HIV cure through CCR5∆32 homozygous allogeneic stem cell transplantation (SCT). Post-SCT events leading to remission in this setting are uncertain, and no detailed analyses of HIV reactivation have been reported. Here we conducted virologic and immunologic analyses of HIV reactivation after CCR5∆32/∆32 SCT.

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were not detected. Three months after ART re-initiation, HIV was detected in plasma (0.39 copies/mL) but not in PBMC (< 0.14 c/mL PBMC). Levels of anti-HIV antibodies remained lower than those detected in PLWH undergoing ART and were comparable to HIV-uninfected controls.

Conclusion: We report the first known case of HIV reactivation during ATI following CCR5Δ32/Δ32 SCT for AML. Our findings indicate that residual RStropic HIV can persist and potentially spread in vivo even >1 year after clinically successful CCR5Δ32/Δ32 allo-SCT.

Figure 1.
**437 VESATOLIMOD PHARMACODYNAMIC RESPONSE IS ASSOCIATED WITH TIME TO HIV REBOUND**

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**Background:** Vesatolimod (VES) is a well-tolerated toll-like receptor 7 agonist that was associated with a modest delay in time-to-HIV-rebound (TTHR) during analytical treatment interruption (ATI) in HIV viremic controllers. This analysis evaluated VES exposure in relation to pharmacodynamic (PD) biomarkers and TTHR, as well as the associations among VES pharmacokinetics (PK)/PD, biomarkers and TTHR.

**Methods:** 25 HIV viremic controllers (pre-ART plasma viral load [pVL] 50-5000 copies/mL) on ART were randomized to receive VES (n=17) or placebo (n=8) biweekly for 20 weeks followed by ATI for up to 48 weeks. Whole blood samples were collected at pre-dose and 24h post first dose to evaluate interferon-stimulated genes (ISGs) with real-time QPCR method. Plasma samples were collected pre-dose* and at 0.5, 1, 2, 4*, 6*, 8, 10*, and 24h after the first dose to evaluate VES PK and biomarkers*. Plasma protein levels were evaluated with multiplex or SIMOA method. Maximum fold change (FCmax) and area under the curve (AUC) of ISGs were analyzed with Spearman’s correlation and Cox proportional-hazards model.

**Results:** Significant increases in VES PK biomarkers and several plasma proteins were observed after the first VES dose and were significantly correlated with VES PK (adj.p < 0.05), including ISGs (ISG15, OAS1, MXI), IFNa, IL-18A, IP-10, CCL4, CCL8, MCP-1, CXCL9, and IL-8. Increased ISGs and CCL4 were associated with longer TTHR. Additionally, Factor VII, MMP3, BDNF, IL-2, and MMP9 were also associated with TTHR. There was no significant association between PK and viral outcomes.

**Conclusion:** These data indicate that VES-mediated biological effects, particularly PD response, were associated with viral outcome in viremic controllers. Larger independent cohorts are needed to validate these findings and investigate the prognostic and functional significance of the biomarkers.

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**438 DIFFERENTIATION ENHANCES REACTIVATION OF HIV-1 IN CD4+ T CELLS AFTER LONG-TERM ART**

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**Background:** Accurately quantifying the frequency of HIV-1 infected CD4+ T cells in people living with HIV (PLWH) on anti-retroviral therapy (ART) remains a significant barrier to evaluation of cure strategies. Molecular measurements cannot discriminate replication fitness of intact provirus, while the Quantitative Viral Outgrowth Assay (QVOA) has been shown to underestimate the viral reservoir frequency. We previously reported that ex vivo differentiation of resting CD4+ T cells to an effector memory phenotype in viral outgrowth assays significantly enhanced HIV-1 latency reversal and demonstrated the replication competent reservoir size was on average 18-fold higher than had been estimated. Here we used the differentiation QVOA (dQVOA) to further refine the relationship between CD4+ T cell differentiation, persistence of the replication competent reservoir and time on ART.

**Methods:** Resting CD4+ T cells were isolated from cryopreserved PBMCs from 32 ART-suppressed PLWH with short (1.0±0.8 years, n=9), intermediate (4.1±0.9 years, n=9), and long-term ART suppression (12.3±3.1 years, n=14). We assessed the frequency of cell-associated (ca-) HIV RNA by real-time RT-PCR, ca-total and integrated HIV DNA by real-time PCR, and infectious units per million (IUPM) CD4+ T cells via both QVOA and dQVOA in parallel.

**Results:** Analysis of the 32 participant samples revealed a relatively stable frequency of cells carrying ca-HIV-1 RNA, ca-total or integrated HIV-1 DNA across the cohort. However, further analysis revealed divergent replication competent reservoir measurements across short, intermediate, and long-term ART suppression. Results from the standard QVOA conditions showed long-term ART suppression was consistent with low IUPM values. In contrast, dQVOA performed on the same pool of resting CD4+ T cells enriched from each participant revealed significantly higher IUPM values from individuals with intermediate (p=0.0056) or long (p<0.0001) term ART suppression. This data suggests long-term ART may not result in a smaller reservoir size, but instead CD4+ T cells from long-term suppressed PLWH may require additional signaling for effective viral reactivation.

**Conclusion:** Together, these data provide insight into the biology of the CD4+ T cells that carry the replication competent reservoir and persist in therapy and suggest that more targeted approaches may be required to reactivate latent HIV after long-term viral suppression, providing crucial insights into designing and evaluating HIV curative approaches.

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**439 A NUCLEOSOMAL MODIFICATION COMPLEX FOR SILENCING HIV**

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**Background:** Post-integration HIV latency is the major hurdle for a cure. To counter HIV latency, it is important to develop a better understanding of the full range of host factors promoting latency. Their identification could suggest new strategies to achieve a functional cure. We recently developed a genetic screening strategy termed “Reiterative Enrichment and Authentication of CRISPRi Targets” (REACT) and unambiguously identified several Silencing Promoting Agents (SPAs) even in the presence of high background “noise” produced by the stochastic nature of HIV reactivation.

**Methods:** In the current study, we modified the REACT method to look for synergies between Silencing Promoting Agents (SPAs) across the human genome. As a proof-of-concept, we used 7 SPAs to conduct 7 parallel screen to look across the human genome for factors that synergize with them to block HIV reactivation.

**Results:** We identified thirty synergies among ten SPAs. Three of them (BCL2L, KANSL2, SIRT2) are previously unrecognized. Overexpression (OE) of BCL2L, KANSL2 and SIRT2 reduced spontaneous and PMA-induced HIV reactivation in J-Lat A2 cells, a Jurkat-based HIV latency model, as well as in CD4 T cells from people living with HIV. These factors inhibit HIV reactivation through different mechanisms: we found OE of BCL2L and KANSL2 increased histone acetylation on the HIV promoter (LTR) region on chromatin, and this in turn resulted in more Brd4S (an inhibitor for HIV transcription) binding to the HIV LTR. In contrast, OE of SIRT2 decreased histone acetylation on HIV LTR. BCL2L is part of the SWI/SNF nucleosomal remodeling complex, while KANSL2 is a regulatory subunit of the KAT8 (MOF) histone acetylation complex. Interestingly, our data demonstrate that BCL2L and KANSL2 are interacting with each other together with a set of proteins from the BAF and NSL subclasses of SWI/SNF and MOF respectively.

**Conclusion:** Our data indicate that a BAF-NSL super complex in the latently infected cells may hyper-acetylate the HIV promoter to recruit excessive Brd4S to inhibit HIV reactivation. Identification and mechanistic characterization of
440 CCNT1 IS A NONESSENTIAL GENE IN JURKAT T CELLS REQUIRED FOR HIV RELEASE FROM LATENCY
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Background: A major barrier to HIV cure is the existence of the latent pool of virus that provides in memory T cells which reactivate upon stopping antiretroviral treatment. We are examining pathways that would prevent reactivation of HIV from latently infected cells with a goal of permanently silencing the latent reservoir. Our hypothesis is that some of the host genes necessary for HIV replication could be non-essential for cell growth but may be key targets for preventing latency reactivation.

Methods: A CRISPR screening method previously developed in the lab called Latency HIV-CRISPR uses packaging of CRISPR guides into budding virions as a readout. We used a library of sgRNAs known as the dependency factor targeting 500 genes involved in HIV replication. Two latently infected Jurkat T Cell lines (Jlats) with integrated provirus were used in this study. Jlats cells were transduced with a lentiviral vector containing Cas9 and a library of guide RNAs targeting HIV dependency factors. Jlats cells were treated with latency reversal agents (LRAs) to stimulate transcription, and viral supernatant and gDNA were deep sequenced to look for guides that were depleted in the supernatant relative to gDNA. The most depleted genes in both cell lines were tested to validate whether they led to a decrease in proviral reactivation on treatment with LRAs.

Results: We find 47 genes from the HIV-DEP library that are depleted relative to gDNA knockout in both Jlat cell lines. The top hit was Cyclin T1 (CCNT1) — a gene known to be critical for HIV transcription. We knocked out CCNT1 with CRISPR and find that it is not essential for host cell growth. However, CCNT1 is essential for HIV transcription and release from latency using several different activators of proviral transcription — including CD3/CD28 and TNFα.

Conclusion: Several HIV dependency factors also play a role in promoting transcription in latently infected cells; thus HIV dependency factors can provide insight into genes that can be targeted to permanently silence the latent reservoir. We find that CCNT1 — a gene that plays both a key role in host and HIV transcriptional reactivation, can be knocked out without dramatic effect on lethality on Jurkat T cells, but dramatic effects on release of HIV from latency. We hypothesize this may be due to the presence of a paralog of CCNT1 — CCNT2, which is involved in host transcription but not required for proviral transcription.

441 ncRNA PROFILE IN EXTRACELLULAR VESICLES REVEALS A POTENTIAL MECHANISM OF HIV-CONTROL
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Background: Recent findings have pointed out extracellular vesicles (EVs) as therapeutic tools against HIV infection given their capacity to transfer HIV restriction factors, including mRNAs and non-coding RNAs (ncRNAs), to nearby cells. However, HIV can also package its own material into EVs to enhance infection and pathogenesis. Herein, we analyzed coding and non-coding HIV RNAs and human ncRNAs content carried by EVs from plasma of patients with different degrees of HIV virologic control.

Methods: Three groups of HIV patients were included according to plasma viral load (pVL): 1) elite controllers (EC), 10 cART-treated patients (TT) and 10 cART-naive with detectable pVL (NT). Ten uninfected controls (UC) were included as reference. EVs, isolated from plasma by Size Exclusion Chromatography, were quantified and visualized by Nano Tracking Analysis and microscopy-TEM. Viral RNAs and human ncRNAs were evaluated by RNaseq in a Miseq System. Identification of RNAs sequences was carried out by mapping them to HXB2 sequence and to human ncRNA database. A differential enrichment analysis of the identified human ncRNAs was conducted with EdgeR, and then, target genes for the differently expressed ncRNAs were predicted with miRDB database.

Results: Compared to EC and TT, Vif(p = 0.03), Vpr(p = 0.03), Pol(p = 0.00) and Gag(p = 0.01) mRNAs, and 3.1TR-RNA(p = 0.03) were more frequently carried in EVs of NT. A total of 109 human ncRNAs were differently expressed between UC and NT, mostly (89%) upregulated in NT. Also compared to UC, only 14 ncRNAs in EC and 9 ncRNAs in TT were differently expressed, mostly (80%) in both cases) upregulated in patients. The comparison between EC and TT showed 28 ncRNAs differentially expressed in 19 ncRNAs upregulated in UC and 9 ncRNAs upregulated in TT. Target prediction showed that ncRNAs upregulated in TT could inhibit expression of HIV restriction factors, whereas ncRNAs upregulated in EC could inhibit expression of cellular targets related to promotion of HIV infection and replication.

Conclusion: Our results show that HIV replication induces the packing of different HIV RNAs in EVs and promotes deregulation in the human ncRNA profile packed in the EVs, which could modify the expression of genes in recipient cells. Moreover, ncRNAs profile differs depending on the viral control (by cART or spontaneous). Of note, EC showed increased levels of ncRNAs that could inhibit HIV replication and enhance immune response against the virus, revealing the importance of EVs as a new mechanism of anti-HIV action.
**443 HIV TEM-SEQ: TARGETED ENZYMIC METHYLATION SEQUENCING OF HIV-1 PROVIRUSES**

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**Background:** DNA methylation is an epigenetic modification that mediates retroviral epigenetic silencing. However, the role of DNA methylation in HIV-1 proviruses remains controversial. Challenges in assessing DNA methylation in HIV proviruses have included large input DNA requirements due to harsh bisulfite conversion methods, low sampling of proviruses and viral regions due to bisulfite PCR sequencing, and problems of designing and optimizing bisulfite PCR primers accounting for HIV sequence diversity. Here, we address these challenges and develop a novel targeted enzymatic methylation sequencing approach termed HIV TEM-SEQ to overcome limitations of prior assays in assessing HIV provirus methylation.

**Methods:** HIV TEM-Seq combines enzymatic methylation sequencing (EM-Seq) and target enrichment of full-length autologous proviral sequences. EM-Seq uses TET2 and T4-BGT to convert 5mc and 5hmc into products protected from deamination from AP0BEC3A. Unmodified cytosines are converted to uracil enabling discrimination and quantification of DNA methylation at single nucleotide resolution. Autologous HIV sequences are utilized for custom capture probe design enriching for unmethylated and methylated proviral sequences spanning the entire provirus. Next generation DNA sequencing provides >30× coverage of all proviral methylation sites.

**Results:** HIV TEM-Seq captured DNA methylation states across the full-length of HIV proviruses in replication competent ACH-2 and U1 cell lines and confirmed increased CpG methylation at the LTR region of single copy integrated provirus in J-Lat 4.8 and S48 latent cell lines. Activation of J-Lat cells with anti-CD3/CD28 and PMA/ionomycin significantly decreased CpG methylation at the LTR region. In proviral DNA from blood and CD4 T cells of people living with HIV, we observed increased non-CpG methylation compared to CpG methylation in HIV proviruses. Confirming prior reports, HIV TEM-Seq did not detect CpG nor non-CpG methylation at LTR regions of in vivo proviruses, suggesting the lack of DNA methylation in the LTR likely promotes the presence of a high transcriptionally active reservoir and permissive latency.

**Conclusion:** HIV TEM-Seq will have future utility in assaying block-and-lock strategies that aim to deposit DNA methylation using epigenetic editing approaches and evaluating whether DNA methylation increases in proviruses of older people living with HIV on long term ART for decades.

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**445 ENHANCED HIV-INDUCED LINEAGE TRACING REPORTER SYSTEM REVEALS EARLY LATENCY PHENOTYPE**

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**Background:** Current Human Immunodeficiency Virus (HIV) treatments effectively suppress viral replication but fail to cure patients of infection due to the persistence of latent infected cells. Latently infected cells are nearly indistinguishable from uninfected cells and evade clearance by the host immune system. Current primary cell models of latency utilize in vitro infection often employ reporter genes that are carried by a replication incompetent HIV. A limitation of these models is that they are susceptible to integration site-specific silencing mechanisms and do not allow tracking of selective pressures of the reservoir driven by pathogenic or immunologic selective pressures.

**Methods:** We have designed an infectious model system that independently reports on a cell’s HIV infection status and viral gene expression and used this model to study the kinetics of infection and early latency establishment. The system employs a cre-lox activated genetic switch, along with replication-competent HIV clone, HIV-miRFP670nano Cre (HIV-RC) that co-express cre-recombinase and miRFP670nano (nano) reporter gene for viral gene expression. This system, described as Enhanced HIV-Induced Lineage Tracing (EHLT), identifies cells with a “history” of HIV infection through an irreversible genetic marking system of HIV-infected cells. HIV infections were performed in T cell lines, primary CD4 T cells from healthy donors, and PBMC engrafted mice (HuPbl mice) with a replication competent HIV designed to express cre and nano as a reporter for viral gene transcription. The system efficiently distinguishes latently infected cells from infected cells with active viral transcription.

**Results:** Infected T cell lines and primary CD4+ T cells with a latent phenotype are established as early as 2 dpi, and with ongoing replication, latent cells accumulate over time. qRT-PCR and DNA qPCR confirmed low levels of viral mRNA expression in latent cells compared to actively infected cells and the presence viral DNA. Higher proviral copy numbers were observed in productively infected cells, while lower copy numbers were observed in surviving latent cells. HIV DNA or RNA was not detected in the unswitched uninfected cells. HuPbl mice infected with HIV-RC showed detectable levels of plasma viremia.

**Conclusion:** This model system enables single cell resolution transcriptional and epigenomic analysis of productive infection verses latent infection in both *in vitro* and *in vivo* settings to probe the forces that shape the latent reservoir.

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**446 TARGETING 2 NEUTRAL SPHINGOMYELINASE 2 PROMOTES DEATH OF HIV-INFECTED CELLS**

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**Background:** Eradication of HIV infected cells is a major goal of cure efforts. Antiretroviral (ARV) drugs suppress HIV, but viral replication rebounds when therapy is discontinued. Efforts to reverse latency are likewise limited by an effective means to kill cells with replicating HIV.

**Methods:** We identified the sphingomyelin hydrolase neutral sphingomyelinase 2 (nSMase2) as a critical component of the late stages of HIV assembly/maturation. We developed phyto(1-3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazol[1,2-b]pyridazin-8-yl)pyrrolidin-3-yl) carbamate (PDDC), a small molecule inhibitor of nSMase2 that is potent (IC50=300nM), selective, with favorable oral pharmacokinetics and safety treatment with PMAI and after 24-hour treatment with PMAI, PEP005 and romidepsin (0.57, 0.37, 0.57- and 0.77-fold change; P < 0.01).

**Conclusion:** Our results suggest that ERα regulation in immune cells is different than what is previously demonstrated in breast cancer. We show for the first time that in CD4+ T cells canonical nuclear translocation of ERα does not occur after exposure to estrogen and that SERD and ERα PROTAC are ineffective at ERα degradation. However, the impact of LRAs on ERα protein levels does not exclude a role of ERα in HIV latency. Collectively, these results suggest that estrogen impact on HIV transcription is unlikely due to canonical ERα nuclear mechanisms of action and merits further investigations.
profile. PDCD was tested in two independent humanized mouse models and mechanisms of action explored in cell culture.

**Results:** The rate of reduction in plasma HIV loads in mice administered PDCD was similar to that achieved with ARVs. With untreated HIV infection the number of CD14+ and CD4+ cells declined as a function of time. ARVs preserved CD14+ and CD4+ cell counts, while PDCD further reduced CD14+ and CD4+ cell counts. CD45+, CD3+, and CD19+ cell counts were unaffected by PDCD. Following drug withdrawal mice administered ARVs exhibited viral rebound, while mice treated with PDCD that achieved plasma HIV below detectable limits (100 copies/ml) did not rebound. An inactive structural analog of PDCD (Cmpd65) had no effect on viral loads or immune cell counts. Results were similar in both models of mice tested.

HIV infection of cells increased nSmase2 expression and its metabolic product ceramide. PDCD or knock down of nSmase2 resulted in oddly shaped, immature, non-infectious HIV particles, enlarged/stressed lysosomes, caspase activation and cell death. The highest dose of PDCD did not reduce the survival of non-infected cells.

**Conclusion:** PDCD is a structurally novel inhibitor of nSmase2 that interferes with the late stages of HIV assembly/maturution. PDCD appears to selectively kill actively replicating HIV infected cells (sparing uninfected cells) resulting in no viral rebound if plasma HIV is reduced to below detectable limits.

**447 DPP9 INHIBITION ENHANCES NNRTI-MEDIATED CLEARANCE OF HIV-1 LATENT RESERVOIRS**

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**Background:** The inflammasome sensor CARD8 can sense intracellular HIV-1 protease activity which leads to targeted cell killing of HIV-1 infected cells which can be achieved using NNRTIs. Additionally, we recently reported that inhibition of DPP9, the negative regulator of CARD8, was able to enhance NNRTI mediated clearance of HIV-1 infected CD4+ T cells in vitro and in humanized mice. However, it’s potential use on clinically relevant HIV-1 strains and its ability to clear latent HIV reservoirs had yet to be investigated. Here we discuss the clinical translatability of this approach for an HIV cure.

**Methods:** CD4+ T cells were isolated from PBMC of healthy donors, infected with a panel of clinical HIV-1 isolates and treated with NNRTIs with or without VbP/1g244 in the presence of T-20 and Raltegravir. Killing was assessed by intracellular HIV-1 p24 staining. To measure removal of HIV latent reservoirs, CD4+ T cells were isolated from people living with HIV (PLWH) under suppressive antiretroviral therapy. These cells were plated in limiting dilution for a quantitative viral outgrowth assay. Cells were treated with either NVP, EFV, or EFV with VbP in the presence of T-20 and Raltegravir. Reductions in the infectious units per million (IUPM) were calculated using the frequency of HIV p24 positive wells via ELISA.

**Results:** We first tested the ability of DPP9 inhibitors VbP and 1g244 to clear replication competent HIV-1 strains from a panel of clinical isolates. We show that both drugs can enhance NNRTI-mediated clearance of infected cells. We also note that 1g244 had lower levels of toxicity and greater activity in vitro.

**Conclusion:** Our previous work on DPP9 inhibition which sensitizes the CARD8 inflammasome laid the foundation for an alternative strategy for eliminating HIV-1 reservoirs. This work expands upon this and shows that this strategy can enhance NNRTI clearance of clinical isolates and reduce viral reservoirs in vivo. This proves the broad suitability of this method for the existing genetic variation seen in PLWH. We also note that other DPP9 inhibitors may be more potent with less off-target effects calling for the development of new DPP9 inhibitors.

**448 INTERLEUKIN-2-INDUCIBLE T-CELL KINASE (ITK) INHIBITION PREVENTS HIV LATENCY REVERSAL**

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**Background:** Inducible T-cell kinase (Itk) belongs to the Tec family of tyrosine kinases, and is expressed in mast cells and T lymphocytes. Itk functions downstream of the T-cell receptor (TCR) and regulates T-cell development, activation and differentiation. The role of ITK signaling in HIV latency persistence is as yet unknown. Here, we determined the impact of ITK on HIV latency and infected cell proliferation in primary CD4+ T cells and cell lines.

**Methods:** CPI-818, a small molecule inhibitor of ITK was used assess the impact of ITK inhibition on TCR signaling by measuring pERK (phosphorylated extracellular signal-regulated kinase) expression by flow cytometry in primary CD4+ T cells, Jurkat and J-Lat 5A8 cells (Jurkat cells latently infected with a GFP reporter virus). To determine impact on latency reversal, J-Lat cells were pretreated with 100 nM, 1 uM, or 10 uM of CPI-818 followed by CD3/CD28 stimulation, and percent GFP positive cells was assayed by flow cytometry. CD4+ T cells from PLWH (people living with HIV) were also used to assess effects on latency reversal. HIV transcripts were quantified using RT-qPCR of Tat/Rev transcripts. The effect of CPI-818 on proliferation of HIV infected CD4+ T cells was assessed in an in vitro infection model by using eFluor670 dye to track cell division. The effect of ITK inhibition on establishment of latency in primary CD4+ T cells was studied in an ex vivo infection model using a dual reporter virus enabling isolation and enumeration of latently- and productively-infected cells.

**Results:** CPI-818 treatment resulted in a significant decrease in the mean fluorescence intensity of pERK in primary CD4+ T cells (p<0.006, t-test) and Jurkat (p<0.002) cells. CPI-818 exhibited dose-dependent inhibition of CD3/CD28-mediated latency reversal in J-Lat cells (p<0.0001). CPI-818 treatment resulted in a significant decrease in latency reversal in primary CD4+ T cells from PLWH (p<0.0001). CPI-818 inhibited proliferation of HIV-infected cells more than uninfected cells (p<0.052). CPI-818 enhanced the initial establishment of latency in primary CD4+ T cells as compared to no-drug control (p=0.0248).

**Conclusion:** ITK inhibition blocks the reversal of latency upon CD3/CD28 stimulation by inhibiting the TCR signaling pathway. ITK inhibition also promotes the initial establishment of latency in primary CD4+ T cells and disproportionately suppresses the proliferation of HIV-infected cells. ITK inhibitors should be explored within antiproliferative and “block-and-lock” HIV cure strategies.

**449 IN-DEPTH ASSESSMENT OF THE HIV-1 VIRAL RESERVOIR IN EARLY TREATED INDIVIDUALS**

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**Background:** The integration site and intactness of proviral genomes remain poorly characterized in early treated individuals on antiretroviral therapy (ART). Moreover, the inducibility of the viral reservoir in these individuals is hampered by the lack of latency reversing agents (LRA) capable of inducing potent HIV reactivation. Here, we did an in-depth assessment of the total and inducible viral reservoir in early treated individuals.

**Methods:** We collected leukapheresis from 8 individuals treated during acute infection (Fiebig II-III: n=6; Fiebig IV: n=1; Fiebig V: n=1) who received ART for a median of 0.96 years (0.49-1.93 years). Total HIV DNA and intact HIV proviral DNA (IPDA) were assessed by multiplex digital PCR. Near full-length (NFL) proviral sequences and integration sites were obtained by matched integration site and IPDA were assessed by multiplex digital PCR. Near full-length (NFL) proviral sequences and integration sites were obtained by matched integration site and IPDA were assessed by multiplex digital PCR. Near full-length (NFL) proviral sequences and integration sites were obtained by matched integration site and IPDA were assessed by multiplex digital PCR.
Conclusion: Collectively, these data indicate that the contribution of clonal expansion to the persistence of the viral reservoir in early treated individuals is minimal after 1 year of treatment. We report a combination of LRAs that allows for the successful detection of the inducible reservoir in early treated individuals on ART. Single-cell assessment of the viral composition of the inducible reservoir should provide further insights into the persistence of those cells during ART.

450 HIV-1 RESERVOIRS IN YOUNG ADULTS AFTER LONG-TERM ART FOR CONGENITAL INFECTION

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1Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 2Bambino Gesu Children’s Hospital, Rome, Italy

Background: Prolonged durations of antiretroviral therapy may allow for immune-mediated selection of HIV-1 reservoir cells, specifically when initiated early after viral transmission. We conducted a deep analysis of the HIV-1 reservoir profile in 9 young adults (age range: 10-26) with congenital HIV-1 infection who started ART at a median of 3.9 months after birth (IQR: 2.3 – 5.8) and remained on antiretroviral therapy for a median of 19 years (range 9 – 26 years).

Methods: Full Length Individual Provirus sequencing (FLIP-seq) was used to interrogate the viral reservoir for the frequency of intact and defective proviruses in peripheral blood samples collected by leukapheresis. Quantitative viral outgrowth assays (QVOA) were used to assess replication competent proviruses. Provilal integration sites were analysed by matched integration site and proviral sequencing (MIP-Seq). Reservoir analyses in young adults were compared to a reference cohort of adults on long-term suppressive ART for a median of 9 years (n=41).

Results: The median number of total HIV-1 DNA copies/10^6 cells and intact HIV-1 sequences/10^6 cells for this cohort (n=9) were 2.96 and 0.02, respectively. In comparison, the median of total and intact proviruses in long-term ART-treated adults were 29.15/10^6 and 2.16/10^6 cells (p<0.0001). In study subject 3, we identified no intact proviral sequences from 300 million PBMC; this result was validated by a QVOA that demonstrated absence of replication-competent virus across 1.5 billion PBMCs/300 million CD4+ cells. In study subject 8, 37 intact proviral sequences were detected across 51 million PBMCs; 19 out of 37 intact sequences were clonal, with an integration site located in the ZNF718 gene on Chr. 4. Subdominant clones of intact sequences were located in the ZNF813 gene on Chr. 19. In study subject 15, no genome-intact proviral sequences were detected across 289 million PBMCs.

Conclusion: In some long-term ART-treated young adults, we noted either complete absence of genome-intact and replication-competent viruses in large numbers of cells, or an integration site profile dominated by intact proviruses in heterochromatin locations. These data suggest that the paediatric immune system can exert effective selection pressure on viral reservoir cells.

451 CTL SELECTION ENRICHES FOR HIV INTEGRATION SITE FEATURES AND EXPANDED CLONES IN VIVO

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Background: In elite controllers (EC) and people on very long-term antiretroviral therapy (ART), the integration sites of intact HIV proviruses become skewed towards transcriptionally inactive genomic loci. Understanding the mechanisms that select for integration sites in gene-distal loci that are refractory to reactivation is key towards developing strategies for functional HIV cures. We devised a novel in vivo model to test whether sustained CD8 T cell pressure would be sufficient to recapitulate these integration site profiles.

Methods: NSG mice were engrafted with memory CD4 T cells from a human clisgender male HLA-B27+ controller with or without autologous CD8 T cells. Viral loads and flow cytometry were performed weekly including HLA-B27+ and KX10 (Gag) tetramer staining. Eight weeks post infection with HIVRCF, human CD4 T cells were quantified in spleens by flow cytometry (Gag+) and the intact proviral DNA assay (IPDA). Proviral integration site isolation and sequencing was performed using ligation-mediated PCR. Integration sites were then identified using the AAvengeR software pipeline. We investigated the association of integration sites with various genomic features, including epigenetic marks, inferred nuclear localization markers, and catalogs of gene expression.

Results: Kr10-specific cells expanded to >20% of CD8 T cells, driving sustained 2-3 log reductions in viral load without immune escape. The ratio of Gag protein-expressing to intact provirus-harboring cells was reduced in +CD8 mice compared to –CD8 mice – consistent with selection for latency (p=0.027). This was associated with a clear footprint on the proviral integration site landscape with enrichment of integrations: 1. Outside of genes (p=0.018), 2. Further away from genes (p<0.001), 3. Closer to the nuclear periphery (p=0.01) and 4. With the provirus in opposite orientation relative the host gene transcription (p=0.002, odds ratio 0.64). The total diversity of integration sites was decimated in the presence of CD8 T cells leaving predominately expanded clones; 10 clones constituted 32-76% of infected cells. No significant differences were observed in the percentages of intact HIV proviruses between +CD8 and –CD8 mice (IPDA, p=0.209).

Conclusion: Our findings validate the idea that CD8 T cells can shape the integration site landscape by selecting for latent proviruses and describe a precise signature of this footprint. This mirrors features seen in ECs and provides guidance for interpreting the impacts of clinical interventions.

452 IMPACT OF HIV RESERVOIR IN THE LOSS OF NATURAL ELITE CONTROL OF HIV-1 INFECTION

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Background: Approximately 25% of elite controllers (EC) eventually lose virological control. This has enabled to classify EC in two groups: i) ‘transient elite controllers’ (TC), those who eventually lose viremia control and ii) ‘persistent controllers’ (PC), those who maintain viremia control indefinitely. It is important to identify the factors that lead to HIV disease progression to open new avenues in HIV cure strategies. Several studies have shown that PC and TC present different immunological, proteomic, metabolomic and miRNA profiles but in terms of virological factors, it is essential to deeply characterize the quality of HIV reservoirs to distinguish both phenotypes.

Methods: Genomic DNA, from 18 PC (viremia control for more than 23 years), 10 TC (sustained viral load above the detection limit; >40 HIV RNA copies/mL, during more than one year of follow-up) before losing the control (0.3-2 years) and 41 antiretroviral-treated individuals for a median of 9 years (2-19 years), was isolated from peripheral blood mononuclear cells (PBMCs). Subsequently, the characterization of HIV-1 reservoir was performed using next-generation sequencing techniques, such as full-length individual proviral sequencing (FLIP-seq) and matched integration site and proviral sequencing (MIP-seq).

Results: PC and TC presented significantly lower total, intact and defective proviruses compared to ART-treated individuals. Although no significant difference was found in total proviruses between PC and TC, a trend in TC to have higher defective provirus was observed (p=0.072). Interestingly, the
proportion of intact proviruses were significantly higher in TC compared to PC (p=0.005). Moreover, non-clonally expanded intact proviruses were found in TC, showing a higher viral diversity in comparison to PC. Regarding the integration sites, intact proviruses from TC and ART were located in permissive genic euchromatic positions in contrast to PC whose intact proviruses were located in centromeric satellite DNA or zinc-finger genes, both associated with heterochromatin features.

**Conclusion:** PC, TC and ART-treated individuals presented a distinct proviral reservoir landscape in PBMCs. The intact proviruses from TC and ART-treated individuals were located in genic regions in contrast to persistent controllers’ intact proviruses that were preferentially integrated in non-genic or pseudogenomic regions.

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**453 DISTINCT INTEGRATION SITES OF INTACT HIV-1 PROVIRUSES IN POST-TREATMENT CONTROLLERS**

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**Background:** For people living with HIV-1, the cessation of combination antiretroviral therapy (cART) typically results in rapid viral rebound. However, a particular group of individuals termed post-treatment controllers (PTCs) are able to maintain undetectable or low viremia for months or years after discontinuation of cART. The mechanisms underlying this control, namely the proviral landscape of PTCs, remain largely unknown.

**Methods:** Samples were obtained longitudinally from 4 PTCs following the discontinuation of cART for up to 4.9 years. Samples were also obtained from 2 individuals who sustained viral rebound following cessation of cART. Genomic DNA was diluted to single-genome levels and then analyzed using full-length proviral sequencing (FLIP-Seq) and matched integration proviral sequencing (MIP-Seq).

**Results:** In total, 2599 proviral genomes were obtained and classified, with 191 found to be intact. Out of the 41 intact proviruses detected in the four PTCs prior to cART interruption, 80.5% were located in non-genic DNA, centromeric DNA or ZNF genes, all of which have been associated with heterochromatin features and “deep” proviral latency. The PTCs also had a relatively high prevalence of intact clonal sequences among total intact sequences (48.3%). Conversely, only a small minority of the n=12 intact proviruses detected in the two rebounders prior to antiretroviral treatment interruptions (ATI) were integrated in non-genic/satellite DNA or ZNF genes (8%, p< 0.0001 relative to PTC). Intact proviruses integrated in non-genic/satellite DNA or ZNF genes prior to ATI persisted at multiple follow-up time points off-therapy; in contrast, the small number of intact proviruses detected in genes prior to ATI were selectively eliminated over time.

**Conclusion:** This analysis suggests that an integration site profile dominated intact proviruses integrated in non-genic or heterochromatin regions of the human genome may facilitate drug-free control after ART discontinuation, presumably because intact proviruses integrated in these regions are in a deeper state of latency and less rebound-dependent. We propose that the integration site profile of intact proviruses can serve as biomarker for identifying putative PTCs and for selecting candidates for future treatment interruption studies.

**454 EFFECTS OF CHEMORADIATION ON EXPANDED PROVIRAL CLONES IN AN ELITE CONTROLLER**

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**Background:** Clonal expansion of latently infected CD4+ T cells contributes to the latent reservoir. Disruption of this process may be needed for viral eradication to be achieved. Recent studies suggest that intact proviruses integrated in certain Zinc finger (ZNF) genes have a survival advantage due to reduced viral expression caused by a repressive epigenetic environment. However, direct evidence of this mechanism is lacking.

**Methods:** We characterized HIV integration sites and used digital PCR assays to measure total, intact, and provirus-specific HIV DNA in an elite controller (EC) before and after he received antiretroviral therapy, chemoradiation, and immunotherapy for metastatic lung cancer. CD8-depleted PBMC were stimulated with overlapping gag peptide for 10 days. Provirus expansion and virus production were used to establish epitope recognition.

**Results:** We detected a marked yet transient contraction in the number of expanded infected-clones after chemoradiation. In addition, there was a modest decline in both total and intact HIV DNA that returned to pre-treatment levels. Of note, his plasma HIV RNA remained undetectable (< 20 copies/mL) throughout the follow-up. We found that the proviral landscape at the end of treatment was dominated by two large clones with replication competent virus integrated into ZNF 721 and 470 genes. One clone, with a provirus integrated in ZNF470, was stable during treatment. In contrast the other clone, which has a provirus integrated in ZNF721 and recognizes the Gag peptide STLQEQIGWMTNNPP (241-255), underwent a 70-fold expansion after chemotherapy was completed. A third clone with a deletion in the primer binding site region and an unknown integration site recognized the overlapping gag peptide for 10 days. Provirus expansion and virus production were used to establish epitope recognition.

**Conclusion:** Our results suggest that chemoradiation can transiently disrupt clonal expansion and can be used in conjunction with other interventions as part
of an HIV cure strategy. Despite the lower inducibility of the ZNF721 integrant, virus production can still occur, suggesting that strong CTL killing is required to maintain elite control of HIV replication.

455 EFFECT OF HIV-1 INFECTION AND VIRUS PRODUCTION ON CD4+ T CELL PROLIFERATION
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Background: The latent reservoir (LR) in resting CD4+ T cells is the major barrier to cure. In HIV-infected individuals on long-term ART, many CD4+ T cells in the LR are clonal and have arisen from proliferation. Consequently, clonal expansion is a major mechanism of HIV persistence. Stimulation of latent-virus-infected CD4+ T cells results in the nuclear translocation of transcription factors that are required for both cellular activation and reactivation of the latent provirus. These two transcriptional programs can result in opposing cell fates, cell division and survival or virion production and cell death. We asked whether proviral intactness, viral particle production, and HIV-1 integration site affect the fate of an individual infected CD4+ T cell.

Methods: We validated a high-throughput assay to isolate individual latently-infected resting memory CD4+ T cells in a background of uninfected cells from peripheral blood of 10 PLWH suppressed on ART for >7 years. Cells were stimulated with anti-CD3/CD28 beads in the presence of IL-2 to mimic antigenic stimulation. ART was included in cell culture media to prevent reinfection. At the end of the culture, clonal expansion and viral particle production were quantified and proviral intactness and integration site were determined.

Results: Uninfected T cells and T cells with a defective HIV provirus proliferated ten and four times better, respectively, than T cells with an intact HIV provirus. HIV-1 integration into a cancer- or proliferation-associated gene endowed a proliferative advantage. Infected cell clones that produced >100,000 viral particles had a proliferative disadvantage. However, viral particle-positive clones did not display inferior proliferation overall compared with viral particle-negative clones. Moreover, >80% of infected cell clones produced no viral particles. Unexpectedly, viral particle production was not strongly correlated with poor infected cell proliferation.

Conclusion: These findings further our understanding of overall reservoir persistence and the fate of individual latently-infected T cells upon antigenic stimulation. Clinically, these experiments suggest that anti-proliferative agents may disproportionately affect uninfected T cells. Although there is no biomarker that distinguishes infected from uninfected T cells, we observed differences in proliferation in response to T cell activation. Future studies should investigate whether dividing infected T cells transiently express viral proteins, which may be targetable.

456 A NOT-SO-STABLE RESERVOIR: HIV DRIVES INFECTED CELLS TO DIE AND DIVIDE
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Background: There is emerging evidence that a significant fraction of HIV proviruses on ART are transcriptionally active resulting in positive and negative selection of infected cells. Evidence of negative selection includes faster decay of intact over defective proviruses while the presence of large proviral clones with integration sites in cancer-related genes is consistent with positive selection. Thus, we hypothesize that HIV drives cells to die and divide faster than their uninfected counterparts.

Methods: To assess the effects of proviral orientation on clone size, we obtained ~3000 integration sites in two chronic progressors at multiple, well-distributed time points over 14 years. We used linear regression and exact binomial statistics to assess effects of proviral integration in the sense orientation. To estimate turnover, we measured clonal overlap between paired time points of proviral sequences by the Morisita index in four chronic progressors and two elite controllers.

Results: Among intrinsic integrations, proviral clones (size ≥1) in sense orientation increase over time more than clones in antisense (p < 0.001). We found small proviral clones favored the antisense orientation, but larger clones increasingly favored sense orientation especially when integrated in cancer-related genes (clone size ≥4: >80% sense, p < 0.05; Figure). We validated this finding with a larger publicly available data set (Coffin et al 2021). Since proviral expression not only increased cell death, but also appeared to increase division, we turned to study turnover. We found proviruses from distant time points had less clonal overlap (p < 0.001) consistent with a reservoir that is turning over. Intriguingly, there was minimal turnover in two elite controllers. Finally, we probed the mechanism behind HIV-host interaction using 40 infected CEM-SS T-cell clones. Surprisingly, the majority of sense proviruses enhanced downstream gene expression by aberrant splicing (median 9-fold increase) while antisense proviruses had no effect.

Conclusion: While reservoir contraction occurs with expression of HIV proteins, expansion is only apparent in sense orientation and frequently results in enhanced downstream gene expression. Sense-oriented proviruses were associated with the largest clones. Intriguingly, proviral turnover appears faster in chronic progressors than elite controllers, consistent with their divergent proviral landscapes in which ECs lack proviruses in sense orientation of cancer-related genes.

Highly expanded proviral clones in cancer-related genes are usually in the sense orientation.

457 ACUTE/RECENT HIV INFECTION IN YOUTH: HIV RESERVOIRS AND ANTIBODY FOLLOWING EARLY ART
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Background: Initiation of antiretrovirals (ART) during acute HIV infection reduces viral reservoirs with improved long term virologic control; there is limited data on youth. HIV reservoir decay in youth treated during acute HIV infection (A) may be greater than in those treated with non-acute infection (NA). ATN 147 identified A or NA HIV-infected youth (12-24 yr) assessing HIV plasma RNA PCR, DNA digital drop (dd) PCR and HIV antibody from baseline (BL) to 24 mo.

Methods: Youth newly diagnosed with HIV infection initiated ART in Los Angeles and New Orleans. Acute infection was defined as Fiebig stage I-V on HIV Western blot (WB) at BL. Fiebig stage VI defined NA infection. Blood was collected over 24 mo. with viral suppression (VS) defined as plasma HIV RNA < 20 copies/ml. HIV DNA ddPCR on PBMC and WB were performed at BL, 12, 24 mo. Statistical comparisons at different timepoints/between groups used log-transformed DNA & RNA values in a mixed effect model.

Results: 103 youth enrolled with mean age of 20.8 yr. (range:16-24); 60% identified as black, 25% Latino, 8% white; 7% Asian/Native American; 90% identified as cis-male, 2% cis-female, 3% trans-female, 3% gender nonconforming; 95% reported same gender/bisexual orientation. ART was initiated within 24 hours in 78%, within 1 week in 88%. At BL, 36 (35%) were A and 67 (65%) NA. Median virus load (VL) at BL was 104,650 (A) and 32,334 cps/ml (NA), p < 0.001 & at 4mo. 30 (A) and 20 cps/ml (NA), p =0.815. By 12 & 24 mo. both A and NA had median VL of 20 cps/ml, p=NS. At 4, 12, 24 mo., 50/76 (68%), 41/57 (72%) youth achieved VS. Median ddPCR copies/million PBMCs for youth over time (BL, 12, 24 mo.) was 844, 192, 127 , p < 0.001. For A and NA had median VL of 20 cps/ml, p=NS. At 4, 12, 24 mo., 50/82 (61%), 41/57 (65%) NA. Median virus load (VL) at BL was 104,650 (A) and 32,334 cps/ml (NA), p< 0.001 & at 4mo. 30 (A) and 20 cps/ml (NA), p =0.815. By 12 & 24 mo. 90% identified as cis-male, 2% cis-female, 5% trans-female, 3% gender indeterminate (NI) WB occurred in 32% of the cohort at 12 & 35% at 24 mo. Negative/indeterminate (NI) WB was 32% of the cohort at 12 & 35% at 24 mo. NI WB was associated with A status at 12 & 24 mo. (OR 11.5; 95%CI:3.88-34.08). VL suppression at 12 & 24 mo. was not associated with NI WB (OR 2.96; 95% CI 0.91-9.66).
Conclusion: Early ART induced sustained VS in 68% of youth by 12 mo., significantly reducing HIV DNA and antibody levels. Within 4 mo. of ART, VS was similar in both A and NA youth. HIV DNA decay was similar in A and NA youth with VS. Acutely treated youth were less likely to maintain positive HIV WB. Median HIV RNA, HIV DNA, and HIV Antibody Responses to Early ART Treatment

Background: The unique features of the infant immune system at the time of reservoir establishment impact the characteristics of long-term virus persistence, susceptibility to immune-mediated clearance, and reactivation potential. These characteristics limit the size of the HIV reservoir which is further reduced over time by a suppressive antiretroviral treatment. These characteristics, coupled with paucity of sample which characterizes the pediatric setting makes the HIV reservoir analysis particularly difficult in perinatally HIV infected children treated early (ePHIV). We here aimed to define the safety of leukapheresis (LA) to deeply characterize the latent reservoir from a large amount of peripheral blood mononuclear cells (PBMCs) in ePHIV that may be considered as candidates for analytical treatment interruption (ATI). We further analyzed RNA seq and DNA methylation in proximity of the intact HIV DNA integration sites.

Methods: nine patients (age range 12-26y, mean age 18,6y), treated within the first year of life (age at ART start=range 0-336 dd, mean 118 dd) with a history of suppressive ART >2 years (range 2-14 years, mean 8,8y) and with a cumulative time on ART ranging from 11 to 25y (mean 17,7y) underwent a LA (avg duration 3,5h). Total CD4, collected from PBMCs collected (range 2,5-9,4 mean 5.06 billions) were analyzed for HIV DNA by Full Length Individual Provirus sequencing (FLIP-Seq) and matched integration and proviral Seq (MIP-Seq). RNA Seq from purified CD4 in unstimulated and stimulated samples (18h PMA and ionomycin) and DNA methylation was performed by Nanopore.

Results: LA was well tolerated in all patients with no adverse events reported. FLIP-Seq showed the total absence of intact provirus in 2 patients where a total of 1,2 billion PBMCs and 280 millions PBMCs were screened. Intact proviruses were detected in 2 patients. RNA Seq and DNA methylation analysis were focused aligning RNA-Seq and DNA methylation in proximity of the HIV proviruses integration sites. These data provided peculiar characteristics both in terms of gene expression after in vitro stimulation and in terms of CpG islands methylation.

Conclusion: This study suggests that LA procedure can be considered a safe procedure for ePHIV where the in depth analysis of HIV reservoir is needed in order to select potential candidates for ATI. The multi OMM analysis within integration sites of intact HIV proviruses may provide crucial information regarding the reactivation potential of the viral reservoir in ePHIV.

Conclusion: LEUKAPHERESIS: A FEASIBLE TOOL TO INFORM ATI THROUGH HIV RESERVOIR STUDY IN CHILDREN

Background: The unique features of the infant immune system at the time of reservoir establishment impact the characteristics of long-term virus persistence, susceptibility to immune-mediated clearance, and reactivation potential. These characteristics limit the size of the HIV reservoir which is further reduced over time by a suppressive antiretroviral treatment. These characteristics, coupled with paucity of sample which characterizes the pediatric setting makes the HIV reservoir analysis particularly difficult in perinatally HIV infected children treated early (ePHIV). We here aimed to define the safety of leukapheresis (LA) to deeply characterize the latent reservoir from a large amount of peripheral blood mononuclear cells (PBMCs) in ePHIV that may be considered as candidates for analytical treatment interruption (ATI). We further analyzed RNA seq and DNA methylation in proximity of the intact HIV DNA integration sites.

Methods: nine patients (age range 12-26y, mean age 18,6y), treated within the first year of life (age at ART start=range 0-336 dd, mean 118 dd) with a history of suppressive ART >2 years (range 2-14 years, mean 8,8y) and with a cumulative time on ART ranging from 11 to 25y (mean 17,7y) underwent a LA (avg duration 3,5h). Total CD4, collected from PBMCs collected (range 2,5-9,4 mean 5.06 billions) were analyzed for HIV DNA by Full Length Individual Provirus sequencing (FLIP-Seq) and matched integration and proviral Seq (MIP-Seq). RNA Seq from purified CD4 in unstimulated and stimulated samples (18h PMA and ionomycin) and DNA methylation was performed by Nanopore.

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458 LEUKAPHERESIS: A FEASIBLE TOOL TO INFORM ATI THROUGH HIV RESERVOIR STUDY IN CHILDREN

459 SIV CLEARANCE FROM NEONATAL MACAQUES FOLLOWING TRANSIENT CCR5 DEPLETION

Conclusion: LEUKAPHERESIS: A FEASIBLE TOOL TO INFORM ATI THROUGH HIV RESERVOIR STUDY IN CHILDREN

Background: The unique features of the infant immune system at the time of reservoir establishment impact the characteristics of long-term virus persistence, susceptibility to immune-mediated clearance, and reactivation potential. These characteristics limit the size of the HIV reservoir which is further reduced over time by a suppressive antiretroviral treatment. These characteristics, coupled with paucity of sample which characterizes the pediatric setting makes the HIV reservoir analysis particularly difficult in perinatally HIV infected children treated early (ePHIV). We here aimed to define the safety of leukapheresis (LA) to deeply characterize the latent reservoir from a large amount of peripheral blood mononuclear cells (PBMCs) in ePHIV that may be considered as candidates for analytical treatment interruption (ATI). We further analyzed RNA seq and DNA methylation in proximity of the intact HIV DNA integration sites.

Methods: nine patients (age range 12-26y, mean age 18,6y), treated within the first year of life (age at ART start=range 0-336 dd, mean 118 dd) with a history of suppressive ART >2 years (range 2-14 years, mean 8,8y) and with a cumulative time on ART ranging from 11 to 25y (mean 17,7y) underwent a LA (avg duration 3,5h). Total CD4, collected from PBMCs collected (range 2,5-9,4 mean 5.06 billions) were analyzed for HIV DNA by Full Length Individual Provirus sequencing (FLIP-Seq) and matched integration and proviral Seq (MIP-Seq). RNA Seq from purified CD4 in unstimulated and stimulated samples (18h PMA and ionomycin) and DNA methylation was performed by Nanopore.

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Conclusion: This study suggests that LA procedure can be considered a safe procedure for ePHIV where the in depth analysis of HIV reservoir is needed in order to select potential candidates for ATI. The multi OMM analysis within integration sites of intact HIV proviruses may provide crucial information regarding the reactivation potential of the viral reservoir in ePHIV.

Conclusion: Early ART induced sustained VS in 68% of youth by 12 mo., significantly reducing HIV DNA and antibody levels. Within 4 mo. of ART, VS was similar in both A and NA youth. HIV DNA decay was similar in A and NA youth with VS. Acutely treated youth were less likely to maintain positive HIV WB. Median HIV RNA, HIV DNA, and HIV Antibody Responses to Early ART Treatment

Background: The unique features of the infant immune system at the time of reservoir establishment impact the characteristics of long-term virus persistence, susceptibility to immune-mediated clearance, and reactivation potential. These characteristics limit the size of the HIV reservoir which is further reduced over time by a suppressive antiretroviral treatment. These characteristics, coupled with paucity of sample which characterizes the pediatric setting makes the HIV reservoir analysis particularly difficult in perinatally HIV infected children treated early (ePHIV). We here aimed to define the safety of leukapheresis (LA) to deeply characterize the latent reservoir from a large amount of peripheral blood mononuclear cells (PBMCs) in ePHIV that may be considered as candidates for analytical treatment interruption (ATI). We further analyzed RNA seq and DNA methylation in proximity of the intact HIV DNA integration sites.

Methods: nine patients (age range 12-26y, mean age 18,6y), treated within the first year of life (age at ART start=range 0-336 dd, mean 118 dd) with a history of suppressive ART >2 years (range 2-14 years, mean 8,8y) and with a cumulative time on ART ranging from 11 to 25y (mean 17,7y) underwent a LA (avg duration 3,5h). Total CD4, collected from PBMCs collected (range 2,5-9,4 mean 5.06 billions) were analyzed for HIV DNA by Full Length Individual Provirus sequencing (FLIP-Seq) and matched integration and proviral Seq (MIP-Seq). RNA Seq from purified CD4 in unstimulated and stimulated samples (18h PMA and ionomycin) and DNA methylation was performed by Nanopore.

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Conclusion: This study suggests that LA procedure can be considered a safe procedure for ePHIV where the in depth analysis of HIV reservoir is needed in order to select potential candidates for ATI. The multi OMM analysis within integration sites of intact HIV proviruses may provide crucial information regarding the reactivation potential of the viral reservoir in ePHIV.

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had shorter duration of time between EDDI and enrollment, and reported more years of education. Individuals in cluster 3 reported fewer years of education, had higher depression scores, and higher HIV viral load at the time of ART onset compared to the other trajectory clusters.

Conclusion: This is the first study to model cognitive trajectories before and after ART among PWH randomized to immediate vs. deferred treatment. Results identify a combination of demographic, psychosocial, and HIV disease variables that contribute to cognitive heterogeneity before ART. Importantly, a short delay in ART onset did not correspond to poor cognitive outcomes at week 192.

Figure 1. Cognitive performances for the three trajectory subgroups

THE COURSE OF NEUROCOGNITIVE PERFORMANCE OVER FOUR YEARS IN WELL-TREATED PLWH

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Background: The Swiss HIV Cohort Study recruited 1,604 PLWH 18 years of age, ≥ 45 years old, who were living with HIV (PLWH ≥ 45 years) at baseline (between 2013 and 2016) and then at two- and four-years follow-up. PLWH 45 years old were recruited and assessed with a comprehensive battery (16 tests) to assess NCI. Demographics, clinical and immuno-virological data from the 2 years preceding and the 2 years following the neurocognitive assessment were collected to identify single (s) or persistent (p) signs of decline. In the current study, we examined neurocognitive performance (NP) after four years, and its associated characteristics.

Methods: The NAMACO study is an ongoing prospective, longitudinal, multicentre and multilingual (French, German, Italian) study embedded within the Swiss HIV Cohort Study. PLWH ≥ 45 years old were recruited and assessed with standardized neuropsychological tests performed by neuropsychologists at baseline (between 2013 and 2016) and then at two- and four-years follow-up. NP was described by Frascati criteria, and by global and per cognitive domains z-scores. To evaluate factors associated with changes in NP over time, we defined our outcome as mean yearly changes in global mean z-scores from baseline. Uni- and multivariable linear regression models were performed and subsequently weighted for the inverse probability of dropping out of the study. Probabilities of dropping out of the study were obtained by multivariable logistic regression adjusted for all baseline characteristics.

Results: At baseline, 981 participants were recruited. A total of 548 (55.9%) completed all three assessments (median age 53, male 80%, Caucasian 96%, MSM 63%, highly educated 89%, smoking 32%, severe alcohol consumption 18%, HIV viral load ≤ 50 copies/ml 97%, and median CD4+ 618 cells/ml). PLWH lost to follow-up were more likely to have ANI at baseline, mild or severe depression, being smokers and to have a moderate or severe alcohol consumption. Neurocognitive trajectories over the study period are presented in Figure 1 (panel A to C). After adjusting for confounders, age over 65 years was the only factor associated with a decline in NP, measured by changes in z-score, after four years (Coeff. -0.03 95% CI -0.05 to -0.01, p-value = 0.005).

Conclusion: In a well-treated population of PLWH we observed stability or improvement of NP after four years of follow-up. Age remained the only factor associated with changes in NP.

NEUROCOGNITIVE IMPAIRMENT NEGATIVELY AFFECTS VIRAL CONTROL IN ART-TREATED PWH

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Background: The clinical relevance of the highly prevalent asymptomatic neurocognitive impairment (ANI) among the spectrum of neurocognitive impairment (NCI) in people with HIV (PWH) is debated. NCI may affect treatment adherence, but very few data are available on whether it significantly affects viral control during antiretroviral therapy (ART).

Methods: Cross-sectional monocentric study enrolling (2010-2019) adult PWH on ART without neuropsychological confounding conditions that underwent a comprehensive battery (16 tests) to assess NCI. Demographics, clinical and immuno-virological data from the 2 years preceding and the 2 years following the neurocognitive assessment were collected to identify single (s) or persistent (p, if 2/s or more consecutive determinations) very low-level and low-level viremia (undetectability < VLLV < 50 cp/mL and 50 cp/mL ≤ LLV < 200 cp/mL), high-level viremia (HLV) and viral failure (VF), when single or 2/s or more consecutive HIV-RNA > 200 cp/mL respectively. Participants on ART since < 6 months before the first collected viremia were excluded. Frascati’s criteria were used to classify NCI severity. Nonparametric tests and logistic regressions were used.

Results: We included 300 PWH: median age and time on ART were 52 and 10 years; 69% male, 96% Caucasian, 85% had undetectable serum HIV RNA at the neurocognitive evaluation; 43.7% and 10.3% were diagnosed with ANI and mild neurocognitive disorders/HIV-associated dementia (M/D). Compared to those without NCI, participants with ANI showed increased risk of pVLLV, sLLV and VF (OR 2.75 p=0.004; 2.31 p=0.041; and 5.34 p=0.003, respectively). The risk increased further for patients with M/D (OR pVLLV 3.08, p=0.024; pLLV 5.24, p=0.001; VF 6.44, p=0.008). Multivariate analysis showed an association between sVLLV and M/D (OR 3.29 p=0.039), pVLLV and M/D (OR 3.08, p=0.024) and both ANI and M/D (OR 2.87 p=0.030) and M/D (OR 8.50 p=0.003), pLLV and M/D (6.40, p=0.016), and between VF and both ANI (7.46, p=0.008) and M/D (9.44, p=0.018), independently from ART type, age, sex, HIV acquisition route, CD4 nadir, calendar year, years on ART, number of plasma determinations and co-medications. PWH experiencing VLLV and LLV had worse scores in tests assessing memory and executive functions only, whereas those developing VF showed broader NCI in several domains.
Conclusion: The whole spectrum of NCI was associated with worse viral control in PWH with a further higher risk of persistent low-level viremia and viral failure in participants diagnosed with symptomatic NCI.

ASSOCIATIONS BETWEEN PLASMA BIOMARKERS AND CHANGES IN COGNITIVE FUNCTION OVER 2 YEARS

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Background: Chronic inflammation may be associated with the development of cognitive disorders. We examine the associations between changes in cognitive function (CF) scores and plasma biomarkers of neuronal injury, systemic inflammation and innate immune activation measured in people with HIV (PWH) and demographically-similar HIV-negative controls in the POPPY study.

Methods: At baseline and 2-year follow-up, participants completed a cognitive test battery assessing psychomotor function, visual learning, visual attention, working memory, verbal learning and executive function. T-scores were derived, with a Global T-score of overall CF obtained by averaging domain T-scores (higher T-score indicates better CF). We used a t-test to assess change in Global T-scores, and linear regression to explore associations between changes in Global T-scores and log-transformed plasma biomarkers (measured at near-fall-up) of neuronal injury (NFL, S100b), systemic inflammation (IL-2, IL-10, TNF-α) and innate immune activation (CD14, IL-10, MCP-1, CD163, MIP-1α) separately. We adjusted for HIV status, age, sex and education; and explored whether effects of biomarkers differed by HIV status using likelihood ratio tests.

Results: 350 participants were included (252 (73%) PWH, median (interquartile range, IQR) age 54 (50-60) years, 85% male, 94% white, and 77% men having sex with men). Among PWH, most (98%) were on antiretroviral therapy, 23% (93%) had HIV RNA ≤50 copies/ml and median [IQR] CD4+ T-cell count was 629 [490, 794] cells/mm3. Mean (standard deviation) (SD) baseline Global T-score was 47.7 (5.9); after median (IQR) follow-up time of 26 [24-29] months, mean (SD) Global T-score was 48.9 (5.5) (p-value for within-individual change <.001). A reduction in CF (i.e. decline in Global T-scores) was associated with higher concentrations of MIP-1α (estimated mean change in Global T-scores: -0.38 [95% confidence interval: -0.61, -0.16] for a 10% increase in biomarker concentration) and CD14 (0.16 [-0.30, -0.02]), though only MIP-1α (-0.46 [-0.68, -0.23]) remained significant after adjustment. There was some evidence that the effects of MIP-1α (p-interaction=.08) and IL-2 (p-interaction=.05) were stronger in PWH (Table).

Conclusion: Within an extensive panel of plasma biomarkers, we observed negative associations with plasma MIP-1α, CD14 and IL-2 suggesting innate immune activation and systemic inflammation may both be implicated in the pathogenesis of CF changes and these effects may differ in PWH.

Table 1. HIV serum parameters, cardiovascular comorbidities, and cognitive test scores at acute HIV (baseline) and week 288 follow-up

VASCULAR AGE AND COGNITIVE OUTCOMES IN AN ACUTE HIV COHORT AFTER 6 YEARS OF ART

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Background: Immune dysregulation persists in people with HIV (PWH) on antiretroviral therapy (ART) and may lead to accelerated vascular aging and cardiovascular (CV) disease. We evaluated relationships between HIV parameters, Framingham Risk Score (FRS)-based 10-year CV risk, vascular age and cognitive function 6 years after ART initiation during acute HIV infection (AHI).

Methods: RV254 Thai participants were enrolled during AHI, initiated ART within days and underwent regular follow-up. FRS-based CV risk at week 288 was calculated using systolic blood pressure, sex, lipid profile, systolic blood pressure, smoking, diabetes and antihypertensive usage status. FRS-based vascular age was defined as age with the same predicted CV risk but optimally-controlled risk factors. Vascular age deviation (VAD) was vascular age minus actual age. Cognitive performance (NPZ-4) was determined by averaging z-scores of: Color Trails 1 & 2, 2-non-dominant hand Grooved Pegboard (GPB), and Trails Making A. Linear regression model was used to assess factors associated with VAD.

Results: The study included 356 participants (98% male; 100% viral suppression) who completed week 288 visits between 5/2009 and 6/2022. CV risk factors are summarized in Table 1. At week 288, the actual and vascular ages were 52 (IQR 38.37) and 34 (IQR 30.40) years (p < 0.001). Vascular age was higher than actual age in 232 (65%) participants (VAD = 31 [IQR-1.7] years). The 10-year CV risk was 2.3% (IQR 1.6,3.9, "low risk" ≤10%). Only one clinically-relevant CV adverse event occurred (embolic stroke) within the study period. In univariable analysis, higher week 288 CD4+ T-cell count was associated with increased VAD (b[95%CI]: 0.5 years [0.3-0.7]) per 100 cell increase in CD4+ T-cell count, p < 0.001. Given a known association between smoking and CD4+ T-cell count in the literature, and that CD4+ T-cell count was 102 (95% CI 20-183) cells higher in smokers (p = 0.015) in this study, stratification analysis was performed. CD4+ T-cell count remained independently associated with VAD regardless of smoking status (p < 0.05). Vascular age and VAD at week 288 were not associated with NPZ-4. There was an unexpected association between higher VAD and better z-GPB scores (b[95%CI]: 0.5 [0.01-1.02], p = 0.045). Conclusion: In young PWH after 6 years of ART initiated during AHI, 10-year CV risk was low and CV events were rare. Higher CD4 count was associated with higher VAD even after controlling for smoking. Vascular risk after 6 years on ART did not predict cognitive test performance.

Table 1. HIV serum parameters, cardiovascular comorbidities, and cognitive test scores at acute HIV (baseline) and week 288 follow-up

465 NON-CLASSICAL MONOCYTES CORRELATE NEGATIVELY WITH HIV-ASSOCIATED COGNITIVE PERFORMANCE

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Background: Despite anti-retroviral treatment (ART), people living with HIV (PLWH) are more susceptible to cerebral small vessel disease (CSVD) and subsequent neurocognitive impairment. Altered blood brain barrier (BBB) and transmigration of inflammatory monocytes are risk factors for developing CSVD. Our group has shown that interaction with activated platelets, drives monocytes towards proinflammatory, promigratory phenotype in vitro. In this study we hypothesized that inflammatory monocytes exacerbate CSVD and cognitive impairment by affecting cerebrovascular permeability and reactivity, especially in older individuals.

Methods: 110 PLWH, on ART and 110 age, sex matched healthy control individuals were enrolled. Platelet-monocyte interaction, monocyte activation...
and soluble markers of monocyte and endothelial activation were measured in whole blood. Neuropsychological testing and brain MRI imaging was also performed. Kruskal Wallis test followed by Dunn’s multiple comparisons and Spearman Correlation were used for data analysis.

**Results:** There was higher prevalence of CSVD (as measured by the presence of white matter hyperintensities on MRI) and cognitive impairment in PLWH. Increased levels of platelet-monocyte complexes (PMCs) were found in PLWH in classical, intermediate and non-classical monocyte subsets. Increased levels of CD14, CD40, PSCG1, TNFR2 and TF were found on PMCs as compared to non-complexed monocytes. PLWH with CSVD had the highest circulating levels of non-classical monocytes as compared with healthy controls with and without CSVD. Percentages of non-classical monocytes correlated negatively with cognitive Z score only in PLWH ($r=-0.2334$, $p=0.0021$), with a much stronger negative correlation in PLWH with CSVD as compared to PLWH without CSVD ($r=-0.3579$, $p=0.0032$). Increased levels of ICAM were found in PLWH, especially those with CSVD and correlated negatively with cognitive Z score ($r=-0.1792$, $p=0.0173$).

**Conclusion:** This study, with a much larger group of study participants, reiterated our previous findings that increased interaction with platelets activates monocytes and drives their differentiation to inflammatory monocyte subset in PLWH. Most interestingly, increased levels of non-classical monocytes can be used as easily measurable predictors of CSVD and cognitive impairment during HIV infection, especially in resource limited settings where MRI imaging might not be available for CSVD diagnosis.

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### 466 COCAINE USE INDEPENDENTLY PREDICTS WHITE MATTER LESION BURDEN IN HIV DISEASE

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**Background:** White matter hyperintensities (WMH), a marker of diffuse small vessel disease and predictor of cognitive decline, has been observed at higher rates in PWH. Aging and cardiovascular disease (CVD) are the main risk factors for developing WMH. The use of cocaine, a potent central nervous system stimulant, is disproportionately common in persons with HIV and may contribute to the development of WMH.

**Methods:** The sample includes 110 adults with chronic HIV disease who were on antiretroviral therapy and had persistent viral suppression. Fluid-attenuated inversion recovery (FLAIR) and T1-weighted anatomical MRI scans were collected, as well as comprehensive neuropsychological testing. FLAIR images were processed using the Lesion Segmentation Toolbox, with a subset of images manually segmented to optimize the threshold for the automatic segmentation. White matter lesion burden was calculated as total lesion volume / total intracranial volume. A CVD risk score for each participant was computed as the sum of common risk factors: diabetes, current smoking, hyperlipidemia, hypertension, obesity, and prior cardiovascular disease, with scores ranging from 0-6. A hierarchical regression model was run to investigate predictors of WMH burden (block 1: demographics; block 2: CVD risk; block 3: lesion burden).

**Results:** The sample was 20% female, 79% African American, with a mean age of 45.37. Mean years since HIV diagnosis was 14.60, and median current CD4+ T-cell count was 750. Nearly a third of participants (29%) were classified as current regular cocaine users, with 23.75 (SD=20.95) days of use in the past 90 days and 18.28 (SD=10.70) years of regular use on average. In the hierarchical linear regression model, after accounting for age, sex, race, and CVD risk, cocaine use was a significant predictor of white matter lesion burden ($\beta=-0.211$, $p=0.036$). CVD risk score also remained a significant predictor in the final model ($\beta=-0.288$, $p=0.003$). Furthermore, in a partial regression accounting for age, white matter lesion burden was negatively correlated with global cognitive function ($r=-0.273$, $p=0.004$).

**Conclusion:** White matter lesion burden is associated with poorer cognitive performance in PWH. Cocaine use and CVD risk independently contribute to WMH, and addressing these conditions as part of HIV care may mitigate brain injury underlying neurocognitive impairment.
EFFECT OF COMMON ANTIRETROVIRAL COMBINATIONS ON DEPRESSIVE SYMPTOMS IN WOMEN WITH HIV

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Background: While modern antiretroviral therapy (ART) effectively suppresses HIV replication, it has been associated with neuropsychiatric adverse events including depression. We examined the combined effect of ART regimens on somatic (e.g. sleep/appetite disturbances) and non-somatic (e.g. sadness) depressive symptoms in women with HIV.

Methods: Women’s Interagency HIV Study (WIHS) participants with ≥2 study visits after January 1st, 2014, receiving common contemporary ART regimens were divided into three groups using longitudinal Center of Epidemiology Studies Depression (CES-D) scale scores: high (CES-D > 16 on >50% of WIHS visits since study enrollment), low (CES-D > 16 on < 50% of WIHS visits), and no (CES-D < 16 for all WIHS visits) depressive symptoms. Novel Bayesian machine learning methods building upon a subset-tree kernel approach were developed to estimate the combined effects of ART regimens on somatic and non-somatic depressive symptoms in each group after controlling for relevant covariates.

Results: Among 1,538 women who participated in 12,924 (mean 8.4) visits, the mean age was 49.9 years; 72.4% were Black, 14.3% were Hispanic, and 72.5% had HIV RNA < 50 copies/mL at study entry. Tenofovir disoproxil fumarate (TDF) (54%) followed by tenofovir alafenamide (TAF) (20%) were the most common ART agents used; 46% received integrase inhibitors (INSTI); 33% non-nucleoside reverse transcriptase inhibitors (NNRTI); and 31%, protease inhibitors (PI). In the high-depression group, the combination of TAF with either a cobalamin-boosted INSTI or PI was associated with greater somatic symptoms (worse concentration, sleep, and motivation) while no difference was observed with TDF in these combinations (Figure 1). Moreover, in the same group, TDF combined with an NNRTI was associated with fewer somatic symptoms. ART regimens were not associated with somatic symptoms in the low- or no-depression groups, and no relationship was found between ART and non-somatic symptoms in any group.

Conclusion: Somatic depressive symptoms were observed more frequently among women who received TAF with a cobalamin-boosted INSTI or PI, but no relationship was found between depressive symptoms and TDF or un-boosted INSTIs or PIs. Our findings suggest complex associations between ART and depression, such that ART combinations rather than individual agents are associated with depressive symptoms. Future studies should consider complete drug regimens when assessing the risk of long-term neuropsychiatric complications of ART.

Combined effects of different ART regimens according to frequency on somatic depressive symptoms by group

470 DTG IMPACTS ZEBRAFISH BEHAVIOUR THROUGH DOPAMINERGIC PATHWAYS: RESCUE BY FOLATE

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Background: Among patients who begin DTG treatment an increase in mild to severe anxiety, insomnia, depression has been observed. Little is known about the mechanisms involved in these symptoms. Zebrafish emerged as an important model for drug screening and neurological research, due to its high similarity with mammals in neuro-anatomical and behavioural features. We explored the locomotor behaviour of zebrafish embryos exposed to DTG and the possibility of a folate rescue.

Methods: Wild type and transgenic (tg:ngn1-EGFP) zebrafish embryos were exposed to 1 µM DTG with/without 5 µM folate from gastrula stage (4 hpf) up to 120 hpf. Locomotor activity was studied by analyzing wild-type larval swimming in a 2 h trial period under dark/light cycling to analyze the embryos’ ability to adapt to environmental stimuli. To understand the relationship between altered behaviour and CNS development, we used tg:ngn1-EGFP embryos, expressing the green fluorescent protein under the promoter regulation of ngn1, a transcription factor playing an important role in the development of dopaminergic neurons.

Results: Embryos treated with DTG had significantly reduced locomotor activity (50% reduction). Folate addition completely restored movement frequency. ngn1-driven fluorescence was evident in central embryos in CNS regions corresponding to the diencephalon (d), hindbrain (hb), ventricle (v) and the trunk region within spinal cord neurons (scn). In treated embryos, ngn1 expression was decreased and impaired in those brain areas particularly enriched with dopaminergic neurons, like (d), (v) and (h), while a consistent number of (scn), peripheral projections of central dopaminergic neurons, were missing, as evidenced by the fluorescence pattern. The addition of folate restored the normal phenotype.

Conclusion: The swimming behaviour of zebrafish embryos in light/dark stimuli is increasingly employed in studying neuroactive drugs. Locomotion in zebrafish is a complex behaviour produced by the activity of various neuronal pathways and its reduction upon the light/dark transition is interpreted as an anxiety state. Our results in the zebrafish embryo model show for the first time DTG effects on the dopaminergic pathways, characterized by a decrease in locomotor activity and an impaired expression of ngn1 in dopaminergic areas of the CNS and in the (scn) of the trunk that can be rescued by folate co-administration.
Dolutegravir Is Associated with More Depressive Symptoms in Older People with HIV

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Background: Integrase strand transfer inhibitors (InSTIs) are first-line therapy in the U.S. and other countries. One InSTI drug, dolutegravir (DTG), has been linked to neuropsychiatric adverse events but few reports have used standardized assessments of depression or assessed the concomitant influence of age and antidepressant use. The aim of these analyses was to determine the relationships between InSTI use, aging, antidepressant use, and depressive symptoms in people with HIV (PWH).

Methods: All 280 participants were comprehensively assessed in the CHARTER Aging project; were taking ART; and had plasma HIV RNA ≤ 200 copies/mL. The project enrolled participants based on chronological age < or ≥ 60 years. The Beck Depression Inventory (BDI)-II and four subscales were compared to biomarkers in a cross-sectional design by multivariable linear regression, including backward selection by Akaike Information Criterion.

Results: Participants characteristics included median age 56 years (28.7% > 60), 18.3% female, 38.8% black, 71.7% AIDS, median CD4+ T-cells 625 μL, median duration of the current ART regimen 19.4 months, and 67.8% InSTI use. Overall, InSTI use was not associated with BDI-II but DTG use trended toward association with worse BDI-II scores (p = 0.064). Multivariable analysis identified that non-black race (p = 0.0033), DTG use (p = 0.0042), female sex (p = 0.011), antidepressant use (p = 0.039), and age ≥ 60 (p = 0.056) were associated with worse BDI-II scores. The effects of DTG were present in the Apathy (p = 0.0027), Cognitive (p = 0.019), and Somatic (p = 0.020) BDI-II subscales. Interaction analyses identified that DTG was associated with worse BDI-II principally among those older than 60 years (interaction p = 0.026, see Figure) and those who were not using antidepressants (interaction p = 0.015).

Conclusion: DTG may increase depressive symptoms in older PWH and those who do not use an antidepressant. DTG did not worsen depressive symptoms in younger PWH or those who were taking an antidepressant. Others have reported neuropsychiatric adverse events associated with DTG but this is the first study to use a standardized depression assessment and to identify an interaction with antidepressants, which may provide mechanistic and clinical insights to improve the care of PWH.
473 SLEEP DISTURBANCES ARE ASSOCIATED WITH COGNITIVE FUNCTION IN WOMEN LIVING WITH HIV

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Background: Poor sleep quality and cognitive impairment are associated with HIV infection; however, the relationship between sleep and cognition in women living with HIV (WLWH) remains underexplored. We examined associations between measures of sleep quality and domain-specific cognitive function in WLWH to identify potential modifiable factors.

Methods: In 2018 and 2019, 337 WLWH enrolled in the Women’s Interagency HIV Study (WIHS) completed the Pittsburgh Sleep Quality Index (PSQI) survey within 12 months of neuropsychological (NP) testing. Linear regression models were used to examine associations between overall sleep quality (total PSQI score 0–21; higher is worse) and NP performance stratified by study-derived cognitive impairment (global T-score < 40 vs. >40), as well as associations between specific potentially modifiable sleep disturbances (e.g., mid-sleep waking, pain, snoring), sleep duration, and sleep medication use, and NP performance. All models adjusted for enrollment site, age, education, race/ethnicity, smoking, drinking, and menopausal status; nadir CD4, log viral load, and body mass index.

Results: Poorer overall sleep quality was associated with worse NP performance among WLWH who had global NP impairment (n=102; Table 1) but not in those without impairment (n=235; P's >0.06). Among WLWH with NP impairment, poorer overall sleep quality was associated with poorer attention/working memory and processing speed; moreover, waking up mid-sleep was associated with poorer processing speed and executive function. Pain disturbing sleep was associated with poorer working memory; snoring was associated with poorer executive function; bad dreams were associated with poorer processing speed; and short (<6 hours) and long (>8 hours) sleep duration was associated with both poorer attention and executive function.

Conclusion: We identified distinct measures of poor sleep associated with diminished performance in several key domains of cognitive function in WLWH. Our findings highlight the importance of targeting sleep disturbances as potential modifiable factors that may influence cognitive performance in WLWH.

Table 1: Results from the multivariable linear regression models examining associations between sleep and cognitive performance among WLWH with global NP impairment

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Memory</th>
<th>Attention/Working Memory</th>
<th>Processing Speed</th>
<th>Executive Function</th>
<th>Motor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Sleep Quality</td>
<td>0.07 (0.00-0.14)</td>
<td>0.06 (0.00-0.13)</td>
<td>0.05 (0.00-0.11)</td>
<td>0.07 (0.00-0.14)</td>
<td>0.06 (0.00-0.13)</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waking up mid-sleep</td>
<td>-0.04 (0.00-0.08)</td>
<td>-0.03 (0.00-0.07)</td>
<td>-0.02 (0.00-0.05)</td>
<td>-0.03 (0.00-0.07)</td>
<td>-0.02 (0.00-0.05)</td>
</tr>
<tr>
<td>Snoring</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal medications last month</td>
<td>0.06 (0.00-0.12)</td>
<td>0.05 (0.00-0.11)</td>
<td>0.04 (0.00-0.09)</td>
<td>0.06 (0.00-0.12)</td>
<td>0.05 (0.00-0.11)</td>
</tr>
</tbody>
</table>

474 BIOPSYCHOSOCIAL PHENOTYPES IN PEOPLE WITH HIV IN THE CHARTER COHORT

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Background: Neuropsychiatric complications such as neurocognitive (NC) impairment (NCI) and depression are common in people with HIV (PWH) despite viral suppression on antiretroviral therapy (ART), but these conditions are heterogeneous in their clinical presentations and associated disability. Identifying novel biopsychosocial (BPS) phenotypes that account for NC performance, depressive symptoms, and daily functioning will promote better understanding of interplay between these conditions.

Methods: We classified 1,580 PWH based on 17 BPS features, including 7 cognitive domains (deficit scores, DDS), 4 subscales of the Beck Depression Inventory-II (BDI-II), 5 components of the Patient’s Assessment of Own Functioning Inventory (PAOFI), and 1 total score of dependence in instrumental activities of daily living (IADLs). A two-stage clustering procedure composed of self-organizing maps (SOM) and k-means clustering algorithms was applied to cross-sectional data. Cluster stability was assessed with adjusted Rand index (ARI) and Cramer’s V. Demographic and clinical characteristics were compared between the clusters using one-way ANOVA and chi-square test for continuous and categorical data respectively.

Results: Four distinct phenotypes were identified: 1) a healthy group (Cluster 1, n=831), 2) a group with mild-to-moderate depression (Cluster 2, n=443), 3) a group with mild cognitive impairment, moderate-to-severe depression, and very impaired daily functioning (Cluster 3, n=191), and 4) a very cognitively impaired group (Cluster 4, n=115). Among WLWH who had global NP impairment (n=102; Table 1), but not in those without impairment (n=235; P’s >0.06). Among WLWH with NP impairment, poorer overall sleep quality was associated with poorer attention/working memory and processing speed; moreover, waking up mid-sleep was associated with poorer processing speed and executive function. Pain disturbing sleep was associated with poorer working memory; snoring was associated with poorer executive function; bad dreams were associated with poorer processing speed; and short (<6 hours) and long (>8 hours) sleep duration was associated with both poorer attention and executive function.

Conclusion: We identified distinct measures of poor sleep associated with diminished performance in several key domains of cognitive function in WLWH. Our findings highlight the importance of targeting sleep disturbances as potential modifiable factors that may influence cognitive performance in WLWH.
serostatus using multivariable linear regression, adjusting for demographics, substance use diagnoses, and relevant co-morbidities.

**Results:** BDI-II scores were elevated in PWH compared to PWoH (11.7±10.8 vs. 6.2±±8.0; p < 0.0001). Two inflammatory factors were identified: Factor 1 loaded on interleukin-6 (IL-6), C-reactive protein (CRP), and D-dimer; Factor 2 loaded on IL-8, chemokine C-C ligand 2 (CCL2), and chemokine C-X-C ligand 10 (CXCL10). Sex modified the effect of Factor 1 on BDI-II, with a more positive association for men than women (β = 0.041). No significant association between Factor 2 and BDI-II was found. Of the biomarkers in Factor 1, only IL-6 was significantly associated with BDI-II and was modified by sex (p = 0.003). In sex-stratified analysis, a positive IL-6 vs. BDI-II association was found for men (β = 5.42; 95% confidence interval = [3.26, 3.57]) but not women (β = –3.78; 95% CI = [-11.02, 3.26]). No HIV-related interactions were detected (see Figure).

**Conclusion:** We identified a depression-associated inflammatory factor present in both PWH and PWoH, consistent with smaller studies of PWH only. The association was driven by a correlation of IL-6 and depression exclusively in men, suggesting that the depression-inflammation link differs by sex. Although associations did not differ by HIV serostatus, PWH may nonetheless be at greater risk for depression in part due to elevated chronic inflammation.

Sex-stratified associations between interleukin-6 (IL-6) and Beck Depression Inventory-II total score, by HIV serostatus.

**476 (NEURO)INFLAMMATORY BIOMARKERS MEDIATE THE ASSOCIATION BETWEEN HIV AND DEPRESSION**

Arish Mudra Rakshasa-Loots1, Nicholas Bakewell2, David Sharp2, Magnus Gisslen3, Henrik Zetterberg4, Jasmini Alagaratnam5, Ferdinand Wit2, Nelfije Kootstra3, Alan Winston2, Peter Reiss2, Caroline Sabin2, Jaime Vera3

COmorBidity in Relation to AIDS (COBRA) cohort

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**Background:** People with HIV are at increased risk for depression, though the underlying mechanisms for this are unclear. In the general population, depression is associated with peripheral and central inflammation. Since HIV infection may elicit (neuro)inflammation, we hypothesised that (neuro)inflammatory biomarkers would mediate the association between HIV and depression.

**Methods:** People with and without HIV in the COmorBidity in Relation to AIDS (COBRA) cohort were included. Using logistic regression, we investigated the possible mediating roles of (neuro)inflammatory biomarkers (neuroimaging, blood, and CSF) on the relationship between HIV status and Any Depression (Patient Health Questionnaire [PHQ-9] scores > 4). We considered changes in the Odds Ratio for Any Depression (OR, adjusted for age, sex, ethnicity, and years of education), before and after adjusting for each biomarker separately. All analyses accounted for varying sample sizes. Biomarkers resulting in >10% reduction in OR were considered potential mediators.

**Results:** We included 204 participants (125 with HIV, 79 without HIV, median [interquartile range] [IQR] age 57 years [51-62], 93% male, 92% White) with PHQ-9 score and at least one biomarker available. Both groups had similar baseline characteristics. All people with HIV were on antiretroviral therapy and had HIV RNA viral load < 200 copies/mL. The prevalence of Any Depression was significantly higher amongst participants with HIV than those without HIV (26.4% vs 11.4%, p = 0.02). The OR (95% confidence interval) for Any Depression in the full sample (N = 204), adjusted for sociodemographic factors but before adjusting for biomarkers, was 3.27 (1.46, 8.09). Of the biomarkers analysed, plasma MIG (-15.0%), plasma TNF-α (-11.4%), CSF MIP1-α (-21.0%), and CSF IL-6 (-18.0%) met our criteria for >10% reduction in OR associated with HIV status for Any Depression.

**Conclusion:** In this sample, MIG and TNF-α in plasma, and MIP1-α and IL-6 in CSF mediated the association between HIV status and depression. As these biomarkers are hallmarks of inflammation (IL-6, TNF-α) and chemotaxis (MIP1-α, MIG), our results implicate different processes constituting (neuro) inflammation in the risk for depression in people with HIV. We did not observe notable attenuation by the neuroimaging biomarkers, suggesting that this mediation may be peripherally driven. Given the lack of gender and ethnic diversity in this cohort, larger and more diverse datasets are needed to extend these findings.

Table 1. OR (with 95% CI) for the association between HIV status and Any Depression, before and after adjustment for each biomarker fitted separately, and percentage change in OR from each model.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>HIV status</th>
<th>Any Depression</th>
<th>Adjusted for</th>
<th>Adjusted for</th>
<th>Change in OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>-11.4%</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>-4.7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-14.5%</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>-4.7</td>
</tr>
<tr>
<td>MIP1-α</td>
<td>-21.0%</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>-4.7</td>
</tr>
<tr>
<td>MIG</td>
<td>-15.0%</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>3.27 (1.46, 8.09)</td>
<td>-4.7</td>
</tr>
</tbody>
</table>

**477 ESTABLISHMENT OF HIV LATENCY AND MICROGLIOSIS IN PRIMARY HUMAN MICROGLIA CELLS**

Eun Hye G. Kim, Roy Missall, Lubberts C. Mulder, Lotje De Witte, Viviana Simon

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**Background:** In the brain, microglia are susceptible to HIV infection and can maintain the viral reservoir. Analysis of HIV latently infected microglia cells at a single-cell level is technically challenging and our understanding of the molecular mechanisms of latency establishment in microglia remains incomplete.

**Methods:** We used pMorpheus-EGFP, a dual-reporter HIV construct, to examine latency establishment in microglia cells isolated from eleven post-mortem human brains. pMorphius-EGFP encodes reporters whose expression is either HIV LTR-dependent (i.e., HSA and Cherry) or independent (i.e., EGFP). Single cycle viruses were pseudotyped with a dual-tropic HIV Env or with VSV-G envelope. Infection of primary human microglia cells were monitored for up to 50 days. We determined the number of productively and latently infected cells by microscopy and by flow cytometry. Proximity extension assay by Olink was used to measure >90 distinct immune-modulatory protein markers in culture supernatants.

**Results:** In contrast to infection of CD4+ T-cells, we found that infection with pMorphius-EGFP resulted in rapid and sustained proliferation of microglia cells (microgliosis). Our results indicate that infection of primary microglia cells at a single-cell level is technically challenging and our understanding of the molecular mechanisms of latency establishment in microglia remains incomplete.

**Conclusion:** Taken together, our findings indicate that infection of primary microglial cells results in rapid and sustained proliferation of infected microglia cells (microgliosis). Further studies are needed to validate these findings in vivo and to identify the mechanisms driving microgliosis.
could contribute to HIV-associated Neurocognitive Disorder (HAND). Treatment would likely require interventions to curb infected cell expansion in addition to limiting productive infections using HAART.

**478 REBOUND HIV-1 IN CSF AFTER TI IS DOMINATED BY CLONALLY AMPLIFIED T CELL-TROPIC VIRUS**

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- University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 1Vitalant Research Institute, San Francisco, CA, USA, 2University of Gothenburg, Gothenburg, Sweden, 3Innsbruck Medical University, Innsbruck, Austria, 4University of California San Francisco, San Francisco, CA, USA, 5Hule University, New Haven, CT, USA, 6Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

**Background:** HIV-1 persists during suppressive antiretroviral therapy (ART) as a latent reservoir but reappears with treatment interruption (TI). In the absence of ART most viral replication takes place within the lymphoid system, although HIV-1 can be detected in cerebrospinal fluid (CSF). Most of what is known about the latent reservoir is based on studies of cells from peripheral blood and less is known about reservoirs in the central nervous system (CNS).

**Methods:** In this study we compare rebound virus in archived blood and CSF longitudinally sampled from 11 participants after TI to look for evidence of a distinct reservoir within the CNS. Single-genome amplification (SGA) and/or Illumina MiSeq deep sequencing with Primer ID were used to assess env diversity and drug resistance in pro-pol. Full-length env genes were cloned from the rebound virus of 4 participants and assessed for their ability to efficiently enter cells with a low surface density of CD4 (a proxy for macrophage tropism; M-tropic).

**Results:** TI occurred a median of 12 years after HIV diagnosis and participants had a median nadir CD4 count of 117 cells/μl. Five participants were suppressed in the blood plasma at the time of TI while 7 participants were viremic. Multiple viral lineages were present in the blood at all time points analyzed after TI. When the CSF lacked ploidy (≤5 WBC/μl), virus in the CSF and blood were genetically mixed and the CSF viral load (VL) was much lower than the plasma VL. At time points with CSF ploidy (>5 WBC/μl), CSF VL reached levels similar to the plasma VL and virus in the CSF was largely clonal. In all participants examined (N=4), CSF rebound virus was adapted to entering CD4+ T cells rather than macrophage (RS T cell-tropic).

**Conclusion:** These results are consistent with a model in which the virus first released after TI in the blood and in the CNS comes from infected T cells. These latently infected T cells, either resident to the CNS or entering from the blood, can proliferate in the CNS and contribute to rebound virus in the compartment. We did not find evidence of M-tropic lineages emerging in the CNS during TI, even in one case where a M-tropic virus was present in the CNS pre-therapy.

**479 IMPACT OF IL-15 NEUTRALIZATION ON CNS PATHOGENESIS IN ACUTE HIV/SIV INFECTION**

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- Beth Israel Deaconess Medical Center, Boston, MA, USA, 1Duke University, Durham, NC, USA, 2Emory University, Atlanta, GA, USA, 3Fredrick National Laboratory for Cancer Research, Frederick, MD, USA, 4Duke University School of Medicine, Durham, NC, USA

**Background:** Immune responses mounted in the CNS during acute HIV/SIV infection are insufficient to prevent viral seeding and reservoir establishment. The pleiotropic cytokine IL-15 plays a crucial role in anti-viral immune response by stimulating NK and CD8+ T cells to control HIV/SIV infection in blood and tissues. However, the effects of IL-15 on the CNS are largely unknown. Experimental neutralization of IL-15, leading to the depletion of NK and T cells, could significantly influence the viral pathogenesis in the brain, after reservoir formation, and clarify the role of IL-15 in lentivirus disease. We comprehensively evaluated brain immune and inflammatory responses to acute SIV infection with and without IL-15 neutralization in an established nonhuman primate model.

**Methods:** Rhesus macaques (Macaca mulatta) were administered two doses of rhesusized monoclonal antibodies against IL-15 (anti-mR-IL-15) at days -21 and -7 prior to challenge with SIVmac239 (day 0) and necropsied at 7 and 14 days post-infection (dpi). A control group did not receive anti-mRIL-15. Peripheral and brain viral load were quantified by qPCR and RNA-seq. Sequencing analysis of viral clones were obtained from several brain regions and compared to those in blood and peripheral lymph nodes. CNS histopathology were analyzed by immunohistochemistry in combination with in situ hybridization. Transcriptomic analyses were performed on brain tissue.

**Results:** While anti-mRIL-15 treatment depleted NK cells from blood and increased virus replication, there was no significant differences in the quantities of SIV RNA or DNA in the brain on either 7 and 14 dpi. Barcoded virus detected in the blood and brain showed clonal expansion restricted to anatomical brain regions. For brain resident cells, peripheral neutralization of IL-15 resulted in increased microglial activation with infection, but decreased the number of astrocytes. While blood T cells were unaltered with IL-15 neutralization, CD8+ T cells decreased in brain, which may have led to an altered balance of local pro- and anti-inflammatory responses, where significantly fewer microglia cells expressed the proinflammatory cytokine – IL-6, and higher numbers of macrophages expressed the anti-inflammatory cytokine – TGF-β.

**Conclusion:** IL-15 neutralization altered CNS immune and inflammatory responses to acute SIV infection by decreasing CD8+ T cells and resulting in a tissue environment favoring anti-inflammation, which could overall support the establishment of viral reservoir.

**480 A FRESH SINGLE-CELL STUDY DETECTS MULTI-COMPARTMENT PERSISTENT HIV RNA DESPITE ART**

Michael Corley\(^1\), Suparnee Bunnapraditkun\(^2\), Alina Pang\(^1\), Alexandra Schuetz\(^1\), Sodsai Tovanabutra\(^1\), Shelli Farhadian\(^1\), Morgane Rolland\(^1\), Nittaya Phanuphak\(^1\), Jennifer Chiarella\(^1\), Carlo Sacdalan\(^1\), Denise Hsu\(^1\), Sandhya Vasan\(^1\), Serena Spudich\(^1\), Lishomwa Ndhlovu\(^1\)

1Wellcome Trust Medical, New York, NY, USA, 2Chulalongkorn University, Bangkok, Thailand, 3Armed Forces Research Institute of Medical Sciences in Bangkok, Bangkok, Thailand, 4US Military HIV Research Program, Bethesda, MD, USA, 5Hule University, New Haven, CT, USA, 6Walter Reed Army Institute of Research, Silver Spring, MD, USA, 7Institute of HIV Research and Innovation, Bangkok, Thailand, 8SEARCH, Bangkok, Thailand, 9Henry M Jackson Foundation, Bethesda, MD, USA

**Background:** Single cell methods enhance the resolution at which infected cells in blood and tissues can be studied in people with HIV (PWH). Capturing the native state of infected cells obtained from multiple tissue compartments, especially cells in the cerebrospinal fluid (CSF), while minimizing cell loss from cryopreservation remains a challenge, especially in resource limited settings.

**Methods:** Fresh blood, CSF, gut, lymph node (LN) and cell-sorted T follicular helper (TFH) cells were collected from participants enrolled in the RV304 SEARCH013 study enrolling people with chronic HIV (PWH) in Bangkok, Thailand where uptake of optional procedures including leukapheresis, lumbar puncture (LP), gut biopsy, and lymph node (LN) biopsy is high. Longitudinal fresh samples from 4 compartments were collected from one PWH at baseline (ART naïve) and after 32 months of ART. An aliquot of baseline CSF cells were set aside and frozen for separate analysis. We used the 10X genomics platform locally to generate single cell RNA and T-cell/B-cell receptor data from fresh cells within hours of sampling and from frozen CSF cells.

**Results:** Single cell data were generated for 18,790 CSF cells, 18,341 gut cells, 37,055 LN cells, 11,561 TFH cells, and 23,349 blood cells. To enhance detection of HIV viral transcripts pre-ART, we generated autologous near full-length patched viral sequences from pre-ART plasma to align single cell sequencing reads and detected high and low HIV transcript containing cells in all compartments pre-ART. Despite 32 months of suppressive ART, transcriptionally active HIV-infected cells were present in all 4 compartments at single cell resolution, including the CNS, predominantly in memory CD4 T cells. Notably, single cell comparisons of fresh (9,603 cells) versus frozen CSF cells (9,187 cells) revealed a significant loss of infected HIV cells in CSF after cryopreservation. T cell clonotypes were shared across CSF, gut, LN, and blood compartments; however, the observed HIV transcript containing cell clones were all unique pre-ART.

**Conclusion:** Longitudinal multi-compartment studies of fresh cells indicate presence of transcriptionally active HIV in several compartments. In CNS, sensitivity was markedly greater in fresh compared to cryopreserved cells. Future studies should evaluate whether HIV-infected cells from diverse tissue sites may be sensitive to cryopreservation, potentially leading to an inaccurate representation of the viral reservoir.
Logistics and workflow of longitudinal multi-compartment single cell study of fresh cells from RV304 in Bangkok, Thailand.

481 INTERACTIONS BETWEEN GUT MICROBIOTA SIGNATURES AND CNS STATUS IN A HIV CURE STRATEGY
Alessandra Borghognone1, Anna Prats2, Bonaventura Clotet1, José Moltó2, Beatriz Martí Puigadas3, Roger Paredes1, Jose A. Muñoz-Moreno1
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Background: The microbiome-gut-brain axis interplay is a major player in regulating the CNS status. In a sub-study from the BCN02 trial, pro-inflammatory microbial signatures were identified as potential predictors of immune-mediated HIV-1 control in the absence of ART. Assessments from a BCN02 sub-study investigating the CNS safety of using the histone deacetylase inhibitor romidepsin (RMD) showed no significant changes in neurocognitive and functional outcomes over the intervention. Here, we investigated possible interactions between the gut microbiota and CNS status in the BCN02 HIV vaccine strategy.

Methods: Associations between microbial abundance (shotgun metagenomics), global neurocognitive functioning (NPZ-6) and functional outcomes (CNS-related symptoms, emotional status, daily functioning, and quality of life) were assessed in 2 subgroups from the BCN02 trial (intervention scheme shown in Figure): (i) HIV-1 viremic controllers and non-controllers (C-NC, n=11) and (ii) intervention and non-intervention (I-NI, n=16) at different timepoints (Figure). Spearman’s correlations and BH-adjusted p-values (≤0.05) were measured by R/rcorr. Gut microbiota profiling in subjects with lower global neurocognitive functioning (NPZ-6< -0.5; n=3) and higher (NPZ-6 >-0.5; n=15) were measured by R/rcorr. Gut microbiota profiling in subjects with lower global neurocognitive functioning were measured by R/rcorr. Gut microbiota profiling in subjects with lower global neurocognitive functioning were measured by R/rcorr.

Results: In the C-NC subgroup, Clostridiales, Methanosaetae stadtmanae and Methanobrevibacter unclassified positively correlated with NPZ-6. Such associations were also observed in the I-NI subgroup, in which methanogenic archaea (Methanosaetae and Methanobrevibacter) as well as short fatty acids (SCFAs)-producing bacterial genera (Megasphaera and Phascolarctobacterium) positively correlated with NPZ-6. Bacteria associated with CNS disorders (CNS-related symptoms, emotional status, daily functioning, and quality of life) were assessed in 2 subgroups. The gut microbiota of subjects with lower NPZ-6 was enriched in brain disorder-associated bacteria and related functions such as S. wadsworthensis and D. desulfuricans (p< 0.05, LDA ≥ 2.0) and 1,2-propanediol degradation pathway.

Conclusion: Neurocognitive functioning positively associates with anti-inflammatory gut methanogenic archaea and SCFAs-producing bacteria in a HIV cure strategy. CNS disruption-associated bacteria are increased in individuals with lower global neurocognitive functioning.

482 ASTROCYTES-MICROGLIA CROSS-TALK: HOMEOSTATIC MODULATION OF HIV EXPRESSION IN MICROGLIA
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Background: The brain cells crosstalk involves a wide range of signaling pathways that can affect gene transcription. We have previously shown that neurons-microglia crosstalk regulates HIV expression in microglia cells. Since the astrocytes are essential homeostatic and immune cells in the brain, we expanded our work to explore the astrocyte-microglia crosstalk in the context of HIV infection. We hypothesize that astrocytes-released factors can regulate microglia activation and therefore silence HIV in infected microglia.

Methods: We used Pluripotent Stem cells (iPSC) derived astrocytes (iA) and the HIV-lentivirus infected microglia cell line H6C9 to identify astrocytes soluble factors regulating HIV. This microglia cell line is an HIV-lentivirus infected clone with a single round HIV-1 containing a short-lived GFP. Cell tracing marked H6C9 cells were co-cultured with iA for 24 hours. GFP expression was determined by microscopy and flowcytometry analysis. H6C9 were also treated with increasing concentration of possible iA-released factors (ATP, BDNF, GDNF, IL10). Coculture of iA and IPCS-microglia (iMG) was established to confirm the results obtained with H6C9. iMG was also infected with the same HIV provirus expressing GFP. Triculture of iA, iPCS-neurons (iN) and iMG was used to confirm regulatory activity of brain cells over HIV-infected microglia.

Results: IA-microglia cocultures showed a significant reduction of HIV expression in microglia cells. We identified that astrocytes-released ATP as responsible for such reduction. Since astrocytes-released ATP can be catabolized to adenosine by CD73, we blocked the CD73 activity with a specific inhibitor (AB-680). The ATP regulatory effect on HIV expression was then diminished, suggesting the effect was indeed due to adenosine and not by ATP itself. Same effect was observed when AB-680 was added to the iA-microglia cocultures. We also observed that low concentrations of ATP (0.5 to 5 mM) would activate the virus. However, with concentrations higher than 10 mM a clear reduction of HIV expression was observed.

Conclusion: IA-iMG coculture and IA-iMG-iN triculture showed that there are in the brain regulatory/homeostatic mechanisms that could be explored to reduce inflammation and HIV expression in infected cells. As our data suggests, one of those mechanisms could be related to ATP/Adenosine. Finally, our results support the importance of studying the crosstalk among different brain cell types to identify possible therapeutic targets.

483 CSF COMPLEMENT IS CORRELATED TO IMMUNE ACTIVATION IN UNTREATED BUT NOT TREATED HIV
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Background: Anti-troviral therapy (ART) has substantially reduced the risk for serious central nervous system (CNS) complications in chronic HIV infection, but milder forms of neurocognitive impairment have been described in people living with HIV (PLWH) using ART. Intrathecal immune activation is a key component in HIV-associated CNS injury in advanced untreated infection, while the importance of the residual, low grade CNS inflammation seen during ART is less clear. The immune activation is initiated by the virus, but secondary immunological mechanisms may contribute to the inflammatory process. The complement system is involved in the pathogenesis of other inflammatory CNS conditions, but the importance in HIV-associated CNS infection, as well as the potential impact of ART is less well characterized. The aim of this study was to explore evidence of complement activation in CSF in untreated HIV infection and in PLWH on ART.
Methods: Retrospective cross-sectional study including PLWH on (HIV-ART) or off (HIV-un untreated) ART monitored in a standardized CSF sampling protocol, and 28 HIV-negative controls (no evidence of CNS infection in clinical lumbar puncture). Complement factor B (CFB), C1q, C3, C4b2a, C5, C5a and C3b was analyzed by commercial ELISA (complement) or immunoassay (neopterin). CSF NfL was analyzed by in-house ELISA.

Results: Forty-five (26 male) PLWH (were included in the study; 17 (9 male) were on ART (plasma HIV RNA < 20 copies/mL). Significant differences in CSF complement concentrations were seen between HIV-un-treated and controls in C1q (p = 0.004), C4b2a (p = 0.003), CFB (p < 0.001), C1b (p < 0.001), C5 (p = 0.001) and C5a (p = 0.02), but not C3a (p > 0.3), between HIV-ART and controls for C1q (p = 0.05), CFB (p < 0.001), C3b (p < 0.001) and C5a (p < 0.001), and between HIV-un-treated and HIV-ART for C4b2a (p = 0.03), C5 (p = 0.02). CSF neopterin was significantly correlated to C1q (r = 0.63; p < 0.001), C3b (r = 0.47; p = 0.01) and C5 (r = 0.52; p = 0.002) in PLWH without, but not on, ART.

Conclusion: Different patterns were found in PLWH compared to controls in CSF complement activity in all activation pathways (classic, alternative, lectin) as well as the terminal cascade. Correlation to macrophage/microglial activation was seen only in untreated PLWH. ART appeared to reduce, but not eliminate, complement activity in CSF. Although the clinical significance of residual intrathecal immune activation needs further study, complement activity may potentially contribute to the CNS pathogenesis in HIV.

484  LONGITUDINAL ASSESSMENT OF GRAPH THEORY MEASURES IN EARLY HIV INFECTION

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Background: Advanced imaging techniques have shown that the brain is comprised of complex networks that work together to support function. Graph theory adapted for neuroscience allows researchers to quantitatively assess connectivity and derive biologically meaningful brain network metrics. In this study, we use resting state functional connectivity with graph theory to evaluate participants in early HIV infection to investigate network changes over the first two years.

Methods: Data from the Chicago Early HIV Infection study was used for this analysis. The cohort included 45 HIV+ (42M, 3F, mean age 32.9 +/- 10.6 years, 30 ART naive, 26 on ART) and 17 seronegative controls (14M, 3F, mean age 31.2 +/- 8.5 years) at baseline. Follow-up data was collected 26.8 +/- 10 months later in ART naïve, 26 on ART) and 17 seronegative controls (14M, 3F, mean age 31.2 +/- 10.6 years, 30ART naïve, 26 on ART) and 17 seronegative controls (14M, 3F, mean age 31.2 +/- 10.6 years, 30

Results:
- Significant differences in network efficiency and clustering coefficient, increased modularity) compared to the control group.
- Path length, which represents the shortest distance between two nodes, is frequently used as a measure for network integration. Our results demonstrate that network efficiency is affected during the early period of HIV infection and that this continues to worsen with infection duration. These findings may provide insight into the development of HIV associated neurocognitive disorder in virally suppressed HIV patients.

Table 1: Global network metrics at baseline and follow up for HIV and control groups. (mean +/- standard deviation. * denotes p<0.001)
CHARACTERIZING THE ROLE OF MONOCYTES IN HIV NEUROPATHOGENESIS

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Background: 15–40% of people with HIV (PWH) develop HIV associated Neurocognitive Impairment (HIV-NCI) despite viral suppression with ART. HIV-NCI is mediated, in part, by transmigration of infected CD14+CD16+ monocytes across the blood brain barrier (BBB), establishing a CNS viral reservoir. We showed that CD14+CD16+ monocytes from PBMC of PWH have increased transmigration across an in-vitro BBB model to CCL2, a chemokine elevated in the CNS of PWH. In a pilot study, we showed that CCR2, the receptor for CCL2, on CD14+CD16+ monocytes is associated with HIV-NCI. This study builds on these findings. Mechanisms by which CD14+CD16+ monocytes mediate HIV-NCI have not been characterized extensively or examined in detail longitudinally. During HIV infection some cells harbor viral DNA (HIV+), were recruited by the Manhattan HIV Brain Bank to undergo 1H-MRS and cognitive evaluation at 2 timepoints, 24 mos apart. PBMC were isolated from participants at both timepoints. PBMC were added to an in-vitro BBB model to analyze CCL2-induced transmigration of monocytes and of HIV+ and HIV- PBMC. We measured associations between transmigration and HIV-NCI status using Frascati Criteria. We evaluated associations betweenCCR2 on CD14+CD16+ monocytes and HIV-NCI. Cross-sectional data from the initial timepoint are presented. We are not powered to stratify by sex. Statistical comparisons were done by Wilcoxon Rank Sum Test.

Results: We showed that CD14+CD16+ monocytes from PWH with HIV-NCI transmigrated more than those from PWH with normal cognition at time 1 (p < 0.05). Transmigration was also higher in those with impairment in speed of information processing (p < 0.05) and working memory (p < 0.01). CCR2 was higher on CD14+CD16+ monocytes in those with HIV-NCI at time 1 (p < 0.05). These results will also be correlated with indices of neuronal health across-the-board at both timepoints. PBMC were isolated from participants at both timepoints. PBMC were added to an in-vitro BBB model to analyze CCL2-induced transmigration of monocytes and of HIV+ and HIV- PBMC. We measured associations between transmigration and HIV-NCI status using Frascati Criteria. We evaluated associations between CCR2 on CD14+CD16+ monocytes and HIV-NCI. Cross-sectional data from the initial timepoint are presented. We are not powered to stratify by sex. Statistical comparisons were done by Wilcoxon Rank Sum Test.

Conclusion: Peripheral circulating CD14+CD16+ monocytes and their CCR2 expression are indicators of CNS dysfunction, even with successful viral suppression.

PLASMA Aβ42/Aβ40 RATIOS ARE NOT REDUCED IN OLDER PERSONS WITH HIV

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Background: As people with HIV (PWH) continue to age, it remains unclear whether they are at higher risk for age-related neurodegenerative disorders e.g. Alzheimer disease (AD), and how to differentiate HIV-related cognitive impairment from AD. This study used a novel blood-based AD biomarker, plasma Aβ42/Aβ40 ratio and the Amyloid Probability Score (APS), in cognitively normal (CN) or cognitively impaired (CI) PWH compared to people without HIV (PWOh) who were CN or had symptomatic AD.

Methods: 66 PWH (age >40 years old) with undetectable viral load (< 50 copies/mL) and 195 PWOh completed a blood draw, magnetic resonance imaging (MRI), and a comprehensive neuropsychological battery or structured clinical interview (Clinical Dementia Rating scale [CDR]). Participants were categorized by degree of cognitive impairment (PWOh-CN n = 43; PWOh_CI n = 23; PWOh_AD n = 57). Plasma Aβ42/Aβ40 ratios were obtained using a liquid chromatography tandem mass spectrometry method. APS, which incorporates Aβ42/Aβ40 ratio, ApoE genotype and age and indicates the likelihood of brain amyloidosis, was also calculated. Log-transformed plasma Aβ42/Aβ40 ratios and the APS were compared among groups. Regression analyses assessed relationships between Aβ42/Aβ40 or APS and hippocampus volume for each group, and between Aβ42/Aβ40 or APS and cognitive domain scores or HIV clinical characteristics (CD4 and CD8 t-cell count, CD4:CD8 ratio, duration of infection) within PWOh.

Results: PWOh_AD (mean log Aβ42/Aβ40= -1.03, SD=0.03; mean APS=41.2, SD=28.9) exhibited significantly reduced plasma Aβ42/Aβ40 ratio (p <.001; Figure 1 and higher APS (p < .001) compared to PWOh_CN (mean log Aβ42/Aβ40 = -0.97, SD=0.03; mean APS=4.73, SD=11.3), PWOh_CN (mean log Aβ42/Aβ40 = -0.98, SD=0.05; mean APS=6.63, SD=13.6), and PWOh_CI (mean log Aβ42/Aβ40 = -0.99, SD=0.06; mean APS=10.2, SD=17.7). There were no differences observed among other groups. Lower Aβ42/Aβ40 ratio and higher APS was associated with smaller hippocampal volume only within the PWOh_AD group. Within the PWH group, neither Aβ42/Aβ40 ratios nor APS were significantly associated with cognitive domain scores, global cognition, or any HIV clinical characteristics (CD4 and CD8 t-cell count, CD4:CD8 ratio, or duration of HIV infection) (p-values > .05).

Conclusion: Plasma Aβ42/Aβ40 ratio and APS may serve as a screening tool for AD and help differentiate cognitive impairment due to AD from HIV-related cognitive impairment in older PWH.

THE CEREBROSPINAL FLUID VIROMES IN VIRALLY SUPPRESSED PEOPLE LIVING WITH HIV

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Background: A relevant proportion of central nervous system (CNS) inflammation and disorders in people with HIV (PWH) remains unexplained despite effective viral control. We aim at describing the cerebrospinal fluid virome (CSFV) of PWH on antiretroviral therapy (ART) and its association with neuroinflammation and CNS outcomes.

Methods: Eighty one CSFVs of adult PWH on three drug regimen ART with plasma HIV RNA< 200 cp/mL and no active CNS infections/disorders were analyzed. Prokaryotic and eukaryotic DNA+RNA were enriched and sequenced following a modified version of QIAseq® Single Cell RNA Library Kit. After host decontamination and background removal, taxonomy and species abundance were estimated by Kraken2 v2.1.2 and Bracken v2.7. The α diversity was estimated by Kraken2 v2.1.2 and Bracken v2.7. The β diversities were estimated by phyloseq v1.8.0 and DADA2 v2.9.2 packages. CSFV composition was compared by the following participants’ features (PFs): demographics, viroimmunological status, mood/neurocognition (Beck Depression Inventory II and 13 neurocognitive tests) and CSF biomarkers (tau, p-tau, neopterin, S100β, Amyloidβ 1-42, intrathecal synthesis, blood-brain...
barrier integrity, cells, proteins, glucose) by Spearman correlations and Mann-Whitney test.

**Results:** Among the 81 participants (71.6% male, median age and CD4+ T cells of 49 years and 455 cell/µL), 25.9% had CSF HIV RNA >20 cp/mL (median [IQR] 46 [30-58] cp/mL). CSFV was not detected in 23 samples, while a median of 305 (122-1035) viral reads was observed in 58 (71.6%) samples. All CSFV+ presented bacteriophages (mostly Siphoviridae, Myoviridae, Podoviridae; 231 [110-1,057] reads), and 7.4% also eukaryotic viruses (EBV, HCV, HHV6, TTV, HPV-96/-201; 122 [79-303] reads). Among all the assessed PFs, CSFV-α diversity was more pronounced in HIV-1 CSF positive compared to HIV-1 CSF negative samples (Fig.A). Higher CSFV-α diversity was also observed in patients with lower CSF glucose and CD4+ T cells count (Fig.B-C). All these associations were confirmed by correlations (p=0.264; 0.444; 0.223, respectively; p < 0.05 for all). No other significant difference in α- and none in β-diversity was observed for any other PFs.

**Conclusion:** Both prokaryotic and eukaryotic viruses were found in the CSF of ART-treated PWH. Our results suggest that worse viroimmunological profiles may correlate with higher CSFV diversity. Further insights are needed to confirm these findings and address the interplay among HIV, CSFV and neuroinflammation.

Alpha diversity of CSF Virome according to CSF HIV RNA, CSF glucose and current CD4+ T cells count

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**CLINICAL AND LABORATORY OUTCOMES OF HEPATITIS C TREATMENT IN AN ACUTE HIV COHORT**

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**RV254/SEARCH O10 Study Team**

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**Background:** HIV/HCV co-infection is associated with impaired immune recovery and cognitive impairment. This study compared the clinical and neuropsychiatric parameters before and after direct-acting antiviral (DAA) therapy with sustained virologic response (SVR) in people with HIV (PWH) on stable antiretroviral therapy (ART).

**Methods:** RV254 cohort participants were enrolled during acute HIV (AHI/ baseline, Fiebig I-V) and initiated ART within days. They underwent assessments at pre-ART baseline, weeks 24 and 96, and every 48 weeks afterwards, including blood tests (complete blood count, liver enzymes, CD4+ & CD8+ T cell counts, HIV RNA, lipid profile and HCV screening), neurological examination, depressive and stress symptoms assessment by Patient Health Questionnaire-9 (PHQ-9, score 0-27) and Distress Thermometer (DT, score 0-10), and a 4-test neuropsychiatric (NP) battery. The battery included Color Trails 1 (CT1) & 2 (CT2), non-dominant hand Grooved Pegboard (GPB), and Trails Making A (TMA). An NPZ-4 score was generated by averaging the z-scores of the 4 tests. To control ART effects, only participants on ≥24 weeks of ART with plasma HIV suppression (HIV RNA < 50 cp/ml) were included. Parameters before and after SVR were compared by Wilcoxon signed-rank test or McNemar's test.

**Results:** Between May 2009 and July 2022, 79 of 688 participants with at least week 24 visit became HCV seropositive; 50 had sustained HCV viremia and received DAA with SVR. All were male with a median age of 30 years (IQR 26-35); 33 (66%) were diagnosed with other sexually transmitted infections within 6 months of HCV seroconversion; 31 (62%) denied any past intravenous methamphetamine use. The durations between AHI and HCV seroconversion and between HCV seroconversion and DAA initiation were 192 [IQR 96-312] and 58 [IQR 27-70] weeks. AST and ALT declined (p < 0.001), and total cholesterol, LDL-C, and triglycerides increased (all p < 0.01) after SVR (Table 1). CD4+ and CD8+ T-cell counts did not change significantly, but CD4/CD8 ratio increased after achieving SVR (p = 0.012). The frequency of peripheral neuropathy and PHQ-9 scores remained unchanged, but stress scores by DT increased after DAA.
491 CMV, SEX, AND COGNITION IN PEOPLE WITH HIV (PWH)

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Background: Interactions between chronic co-infections, HIV persistence, and immune response have been implicated in persistent inflammation and cognitive disorders in people with HIV (PWH), but findings have been inconsistent. Most data are based on serology and have not examined sex effects. We aimed to assess the relationships among viral DNA, IgG, and cognition and whether they are influenced by assigned sex at birth.

Methods: Participants included 486 PWH (81 female) who had prior comprehensive cognitive testing, HIV RNA < 200 copies/mL on antiretroviral therapy and stored blood. Digital droplet PCR quantified CMV, EBV, and total HIV DNA measured in plasma by commercial immunoassays in a subset (N=267 and 300, respectively). Using linear regression, we tested the association of viral DNA, IgG, and cognitive disorders in people with HIV (PWH), but findings have been inconsistent. Most data are based on serology and have not examined sex effects. We aimed to assess the relationships among viral DNA, IgG, and cognition and whether they are influenced by assigned sex at birth.

Results: CMV DNA was detected in 45.4% and EBV DNA in 95.5% of samples. Overall, lower CMV DNA levels tended to associate with worse Global cognitive T score (p=0.0045, NPZ 4 (p=0.0044) and z-MA (p=0.028) improved significantly post-DAA. Of note, no improvements in CD4/CD8 ratio and NP battery were observed between HCV serocconversion and DAA initiation.

Conclusion: In PWH on stable ART with HCV co-infection, CD4/CD8 ratio and cognitive test performance improved after DAA therapy with SVR. Laboratory and clinical outcomes of participants who achieved sustained virologic response (SVR) after DAA therapy (N=50).

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492 POST-COVID COGNITIVE IMPAIRMENT MRI BRAIN SCAN ABNORMALITIES: A POTENTIAL BIOMARKER

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Background: Post-acute sequelae of SARS-COV-2 infection (PASC) is associated with cognitive impairment (CI) with unclear pathogenesis though blood brain barrier (BBB) impairment and excitotoxic injury appear significant.

Post-acute sequelae of SARS-COV-2 infection (PASC) is associated with cognitive impairment (CI) with unclear pathogenesis though blood brain barrier (BBB) impairment and excitotoxic injury appear significant. We hypothesized that PASC CI patients would have brain inflammation and BBB disruption using advanced MRI imaging.

Methods: In this prospective longitudinal study, 14 patients with PASC CI (mild and non-hospitalised) were enrolled (mean age of 45; 10 F and 4 M) and 10 sex and age matched healthy controls. 13 had a follow up MR at 9-12 months (mean 10 months). All participants underwent DCE perfusion (an index of BBB integrity with Ktrans as the measurement), Diffusion Tensor Imaging (DTI) and single voxel MR spectroscopy (MRS) of the frontal cortex/white matter and the brainstem in addition to brain anatomical MRI. Between group analyses were used to determine which MRI abnormalities were significantly different from controls in patients with PASC CI.

Results: The PASC CI group showed significantly increased (ie BBB impairment) Ktrans, and increased radial and mean diffusivity. Increased Ktrans was correlated with increased both radial and mean diffusivity. Reduced Ktrans significantly improved in the follow up MR (p = 0.00004) with no difference between subjects and controls indicating BBB normalisation (p = 0.44218, z = -0.144841). White matter integrity also improved especially in all tested brain regions. Ktrans significantly improved in the follow up MR (p = 0.02596, z = -2.794872) with no difference between subjects and controls indicating BBB normalisation (p = 0.00004). MRS showed significant improvement in the NAA in the frontal white matter but Glx remain high as compared to the controls (p = 0.00006).

Conclusion: PASC CI patients would have brain inflammation and BBB disruption using advanced MRI imaging.

493 COGNITIVE, MOTOR, & NEGATIVE VALENCE SYSTEMS IN NEUROLOGIC PASC: A PRELIMINARY STUDY

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Background: Nervous system post-acute sequelae of COVID-19 (NS-PASC) include cognitive and mental health symptoms. To further define these, we applied a Research Domain Criteria (RDQ) approach to examine motor, positive valence (PV) and negative valence (NV) systems, and social processing data in The COVID Mind Study of NS-PASC.
CHARACTERISTICS AND OUTCOMES OF COVID-19 IN AN EARLY-TREATED HIV COHORT IN THAILAND

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Background: Emerging data indicate that people with HIV (PWH) are at risk of more severe outcomes from COVID-19. We described the clinical course and laboratory parameters pre- and post-COVID-19 in an early-treated HIV cohort in Thailand.

Methods: RV254 cohort participants were enrolled during Fiebig I-v Acute HIV and initiated antiretroviral therapy (ART) within days. They underwent regular blood tests (CD4+ & CD8+ T-cell counts, HIV RNA), neuropsychiatric (NP) assessment (Color Trails 1 & 2, non-dominant hand Grooved Pegboard, Trails Making A), and mood questionnaires (Patient Health Questionnaire-9, Distress Thermometer) post-enrollment longitudinally. Their assessment outcomes pre- and post-COVID-19 were compared using Generalized Estimating Equations (GEE) with a normal distribution and identity link (CD4+, CD8+ T-cell counts, NP parameters) or binomial distribution with log link (HIV RNA), and autoregressive correlation structure.

Results: Between 4/2021 and 9/2022, 295 participants on ART (98% male, median age 32 [IQR 28-37]) were diagnosed with COVID-19. Of these, 165(55%), 38(13%) and 241(82%) were infected with α, δ and o variants, determined by median age 32 [IQR 28-37] were diagnosed with COVID-19. Of these, 16(5%), 0(0%), and 223(75%) were admitted to hospital. A total of 123 participants were managed in hospital or ‘hospitel’, including 106 (36%) were managed in hospital or ‘hospitel’, including 38(13%) and 241(82%) were infected with α, δ and o variants, determined by median age 32 [IQR 28-37] were diagnosed with COVID-19. Of these, 16(5%), 0(0%), and 223(75%) were admitted to hospital. A total of 123 participants were managed in hospital or ‘hospitel’, including median age 32 [IQR 28-37] were diagnosed with COVID-19. Of these, 16(5%), 0(0%), and 223(75%) were admitted to hospital. A total of 123 participants were managed in hospital or ‘hospitel’, including median age 32 [IQR 28-37] were diagnosed with COVID-19. Of these, 16(5%), 0(0%), and 223(75%) were admitted to hospital. A total of 123 participants were managed in hospital or ‘hospitel’, including
496 INFLAMMATORY AND BLOOD-BRAIN BARRIER MARKERS AFTER COVID-19 AMONG PEOPLE WITH HIV

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Background: COVID-19, the disease caused by SARS-CoV-2, has resulted in devastating morbidity and mortality worldwide. Alarming evidence indicates that long-term adverse outcomes of COVID-19 can affect all major systems of the body, including the immune, respiratory, cardiovascular, and neurological systems. While acute COVID-19 pathology does not appear to be markedly different by HIV status, long-term outcomes of COVID-19 in People with HIV (PWH) are unknown and require further investigation. This study evaluates inflammatory profile longitudinally up to three months after COVID-19. In addition, markers of the blood-brain barrier (BBB) integrity and vascular dysfunction were also evaluated.

Methods: Plasma samples were collected from 15 males and 6 females with COVID-19 and HIV infection (COVID+/HIV+) and 9 males and 14 females with COVID-19 without HIV infection (COVID+/HIV-) between March 2020 and March 2021. Baseline samples were obtained approx. 10 days after COVID-19 diagnosis (T=0) and three months after (T=3). Mean age group for COVID+/HIV- was 52.1±12.3 for males and 48.7±11.6 for females (N=15 and 6, respectively). 27 out of 27 inflammatory analytes, 20 had detectable signals. Eotaxin (CCL11) and G-CSF levels were differentially upregulated in the COVID+/HIV+ group as compared to COVID+/HIV- group in both time point studied (Table 1). IFN-γ showed sustained increased levels at T=3 in the COVID+/HIV+ group, whereas there was a significant reduction over time in the COVID+/HIV- group. At T3, inflammatory markers (IL-4, IL-8, IL-13, basic FGF, TNF-α, MIP-1α, and CCL2) either decreased or remained unchanged in both groups. In contrast, the markers of the BBB disruption and vascular dysfunction, such as soluble ICAM-1 increased in the COVID+/HIV+ group, suggesting long-term progressive BBB and vascular alterations.

Conclusion: Neurocognitive scores in PWH were independent of COVID status, suggesting that higher frequencies of post-COVID neurocognitive dysfunction in PWH compared to HIV-seronegative people are due to HIV-associated factors more so than COVID. HIV-seronegative, post-COVID people demonstrate diminished recognition memory, processing speed, and executive function at 1 month post-COVID that improves by 4 months. Post-COVID neurocognitive dysfunction is present, if temporary, even in a highly vaccinated cohort of people.

497 MODELING TO OPTIMIZE ISLATRAVIR DOSE IN HIV VIOLENTLY SUPPRESSED PWH

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Background: Islatravir (ISL) is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) being studied for HIV-1 treatment and prevention. Exposure-related decreases in total lymphocytes and CD4+ T-cell counts were observed across ISL clinical trials, with higher frequencies and magnitude of changes observed in ISL higher-dose regimens (20 mg once weekly (QW); 60 and 120 mg once monthly). Data from Phase 2 and 3 ISL treatment and PrEP trials were used to develop models that describe the changes in lymphocytes and CD4+ T-cells in relationship to intracellular ISL-TP concentrations. Optimized doses were identified to achieve efficacy thresholds and similar CD4+ T-cell and lymphocyte dynamics compared to standard antiretroviral therapy (ART).

Methods: An ISL popPK model was developed incorporating ISL PK data from once daily (QD) and QW doses. Subsequently an ISL popPKPD model was developed incorporating longitudinal CD4+ T-cell and lymphocyte data from long-term ISL studies. Additionally, CD4+ T-cell changes were summarized across approved ART regimens for the virologically suppressed population to compare to PK/PD model predictions. Revised ISL QW doses were selected based on simulated doses providing ISL exposures ensuring coverage for WT and M184V variants as well as CD4 and Lymph counts changes comparable to standard ART in switch population.

Results: The ISL population PK model was updated with additional phase 1 studies and the Phase 3 QD HIV trials and captures both plasma ISL and intracellular ISL-TP dynamics across regimens. The CD4+ T cell and lymphocyte models were developed and captures the changes for treatment naive, virologically suppressed, and prevention populations on standard therapy. The CD4+ T cell model captured the ISL changes in ISL in the daily treatment Phase 2 and 3 studies and the lymphocyte model additionally captured the ISL changes for the Phase 2 PrEP trial. The summary of switch trials show that the majority of trials have CD4+ T cells changes that fall between -5 and +10% changes over the course of the trials and allowed for benchmarking of the CD4+ T-cell model simulations. Simulations of the models were conducted for ISL 0.5-20 mg QW predicting ISL-TP exposures and CD4+ T cell and lymphocyte changes.

Conclusion: ISL 2 mg QW is predicted to rapidly achieve efficacious exposures for wild-type and M184V HIV/III variants and have similar CD4+ T-cell and lymphocyte changes as standard ART as virologically suppressed PWH.

498 ESTRADIOL CONCENTRATIONS IN TRANS WOMEN ON INSTIS COMPARED TO THOSE WITHOUT HIV

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Background: Accessing feminizing hormone therapy (FHT) is essential to trans women. Concern of negative drug interactions between their FHT and antiretroviral therapy (ART) can be a barrier to acceptance of ART in trans women.

Results: Of 27 inflammatory analytes, 20 had detectable signals. Eotaxin (CCL11) and G-CSF levels were differentially upregulated in the COVID+/HIV+ group as compared to the COVID+/HIV- group in both time point studied (Table 1). IFN-γ showed sustained increased levels at T=3 in the COVID+/HIV+ group, whereas there was a significant reduction over time in the COVID+/HIV- group. At T3, inflammatory markers (IL-4, IL-8, IL-13, basic FGF, TNF-α, MIP-1α, and CCL2) either decreased or remained unchanged in both groups. In contrast, the markers of the BBB disruption and vascular dysfunction, such as soluble ICAM-1 increased in the COVID+/HIV+ group, suggesting long-term progressive BBB and vascular alterations.

Conclusion: HIV-1 may potentiate long COVID-19-induced neuropathology, with progressive BBB breakdown and sustained increase in eotaxin-1 and G-CSF. Plasma inflammatory markers in COVID-19 patients with or without HIV-1 co-infection

Table 1. Plasma inflammatory markers in COVID-19 patients with or without HIV-1 co-infection
women with HIV. In this study, we measured serum estradiol concentrations in trans women with HIV taking FHT and integrase strand transfer inhibitor (INSTI)-based ART versus trans women without HIV taking FHT.

**Methods:** This was a single-center, parallel group, clinical trial. A total of 32-50% women with and without HIV, 18 years or older, and taking at least 2 mg/day of oral 17-beta estradiol plus a form of anti-androgen therapy, with no medication changes for at least 1 month prior to inclusion. Women with HIV were to be on suppressive ART. Blood was collected prior to ART and estradiol dosing and then at 2, 4, 6, and 8 hours post-dose and estradiol concentrations were measured from serum using CMA (Lifelabs). Median estradiol concentrations at each time point, estradiol \( \text{Cmax} \), and \( \text{Tmax} \), were calculated and compared between groups using Wilcoxon rank-sum tests.

**Results:** Participants \((n=15)\) were enrolled March to August 2022 and had a median age of 32 (IQR: 28-39); 8 participants with HIV had a median age of 36 (IQR: 32, 48.75) years compared to 30 (IQR: 27.25, 41.75) years for 7 participants without HIV \((p=0.042)\). Among trans women with HIV, the median duration of HIV was 9.5 years \((5.0, 23.0)\); 6 were taking bictegravir/emtricitabine/tenofovir alafenamide and 2 were taking dolutegravir/abacavir/ lamivudine. Among all participants \((n=15)\), the median oral estradiol dose was 4 mg \((range 2-6 mg)\). Anti-androgen therapy (some on multiple) included spironolactone \((n=8)\), orchidectomy \((n=6)\), central hypogonadism \((n=1)\), and cyproterone \((n=1)\). Three participants were taking progesterone. Participants had been taking FHT for a median of 4 years \((2, 8)\). Eleven \((73\%)\) participants had ideal estradiol concentrations of 200 to 735 pmol/L at C4h \((75\% among women with HIV and 77\% among those without HIV). Table 1 summarizes oral estradiol concentrations overall and by HIV status. No statistically significant differences were identified by HIV status.

**Conclusion:** In trans women on FHT, estradiol concentrations were similar between trans women on ART and trans women without HIV with a slightly higher \( \text{Cmax} \), among those with HIV. This suggests a low probability of clinically relevant drug-drug interactions between FHT and INSTI-based ART.

Table 1: Summary of oral estradiol concentrations overall and by HIV status

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HIV status</th>
<th>( \text{Cmax} ) (nmol/L)</th>
<th>( \text{Tmax} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-21</td>
<td>HIV+</td>
<td>200-735</td>
<td></td>
</tr>
<tr>
<td>22-30</td>
<td>HIV+</td>
<td>200-735</td>
<td></td>
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</tbody>
</table>

**Figure 1:** Serum Concentration of N6LS. Geometric mean serum concentrations with standard deviation (indicated by bars) are depicted for each study group with single administration of N6LS with or without rHuPH20. The dose, administration route, and route of administration for each group is specified in the key. SC denotes subcutaneous route of N6LS administration.

**TFV-DP & FTC-TP IN BLOOD CELL SUBSETS OF PERSONS WITH HIV**

**Vincent A. Mainella**

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**Background:** Nucleos(t)ide reverse transcriptase inhibitors (N(t)RTIs) are activated to their active forms within multiple cell types, but data have been primarily focused on red blood cells, dried blood spots (DBS) and peripheral blood mononuclear cells (PBMCs) to date. Understanding the extent to which N(t)RTIs accumulate in other major blood cells may inform clinicians’ understanding of ART efficacy and toxicity, such as cardiometabolic effects. We examined the cell pharmacology and platelet effects of abacavir (ABC)/lamivudine (3TC) and tenofovir alafenamide (TAF)/emtricitabine (FTC) in persons with HIV (PWH). Here, we aimed to establish TFV-diphosphate (DP) and FTC-triphosphate (TP) concentrations in different cell types and compare them with historical data in persons without HIV (PWOH).

**Methods:** PWH receiving TAF 25mg/FTC 200mg as part of clinical care with HIV VL < 200 c/mL for ≥6 months prior to entry were eligible (NCT04301661). Adherence was confirmed using video directly observed therapy (vDOT) for 28±3 days prior to PK sample collection (2±1 hrs post-dose). Whole blood was processed into DBS, PBMCs, platelets, and neutrophils. LC-MS/MS methods were used to quantify TFV-DP and FTC-TCP across all cell types in addition to the mono (MP)- and DP fractions in platelets. Data in PWOH originated from TAF-DBS (NCT02962739). Cellular concentrations by HIV status were compared using linear regression models.

**Results:** 13 PWH were enrolled in the TAF/FTC cohort (12 male; 8 white, 2 black, 3 other; 4 Hispanic/Latin; median [range] age and weight of 42 [26-64] years and 74.2 [58.3-116.7] kg). Median (range) 4-week vDOT adherence was 96% (76-100%). Historical data were available in 31 PWOH (median [range] age 29 [18-41] years and weight 76.3 [56.7-100.2] kg). Cellular concentrations by cell type are summarized in the table. Within platelets, TFV-DP > TFV-TP > FTC-DP > FTC-MP. TFV-DP in DBS and platelets were 73% higher and 61% higher, respectively, and FTC-TCP in platelets were 71% higher in PWH vs. PWOH. No other differences were identified.

**Conclusion:** Higher TFV-DP in DBS is consistent with previous studies in PWH, but higher TFV-DP and FTC-TCP concentrations in platelets suggests an undescribed difference in cell-specific physiology in PWH. The mechanism behind this differential accumulation warrants further investigation. Cellular data with ABC/3TC and comparisons of metabolic effects in platelets may help provide further insight into intracellular processes.
TAF-FTC Anabolites by Cell Type in PWH & comparisons to PWOH

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>TAF-DP (µM)</th>
<th>Percent Difference (µM)</th>
<th>TFC-TF (µM)</th>
<th>Percent Difference (µM)</th>
</tr>
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<tbody>
<tr>
<td>C24h/3do (µM)</td>
<td></td>
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<tr>
<td>C24h (µM)</td>
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<tr>
<td>C72h/3do (µM)</td>
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501 IN SILICO PHARMACOKINETICS EVALUATION OF FORGIVENESS FOR DORAVIRINE & RILPIPVINE

Yeelen Fromage, Najwa Jamal, Cyrielle Codde, Jean-François Faucher, Jean-Baptiste Woillard

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Background: ANRS 170 QUATUOR findings showed the non-inferiority of some intermittent maintenance treatment strategies, but treatment failures related to emerging disease-resistant mutations with rilpivirine as third therapeutic agent have been observed. The consequences of the 3-days-off strategy on non-nucleoside reverse transcriptase inhibitors (NNTI) concentrations are not known.

The aim of this study was to evaluate in silico, using simulation from population pharmacokinetics models, the concentrations of rilpivirine (RLP) after 3 days off, with doravirine (DOR) as a comparator.

Methods: Previously published population pharmacokinetic models for DOR (Yee et al, Antimicrob Agents Chemother. 2019) and for RLP (Aouri et al, Antimicrob Agents Chemother. 2016) were implemented in the mr_solve R package. 10000 Monte Carlo simulations at steady state for typical dose of 25 mg/day for RLP and 100mg/day for DOR were drawn for two scenarios: without drug cessation and after 3 days off. Filters were applied on simulated trough concentration (C24h) to keep the 2.5 to 97.5 simulated profiles. The validation of the implementation was performed based on the comparison of the median C24h to the one observed in the literature. The proportion of simulated patient with C24h and C72h after 3 day off (C72h/3do) higher than IC50 (5.2 µg/L (DOR) ; 20.5 µg/L (RLP)) and IQ (6 x IC50 (DOR); 4.5 x IC50 (RLP)) were calculated for both drugs. Finally, nomograms to estimate probability of having a concentration > IC50 after 72h of drug cessation for different range of C24h were built.

Results: Simulated C24h median range [RPL] for RLP were 62.8 [23-142] µg/L and for DOR 400 [209-603] µg/L. The proportion of patient with C24h > IC50, C72h/3do > IC50, and C72h/3do > IQ were 100%, 36.6% and 0% for RLP and 100%, 89.4% and 74.3% for DOR. The nomogram for probability of target attainment at 72h as function of C24h range is presented in Table 1.

Conclusion: Based on these findings, treating with DOR would be more forgiving than with RLP since the former drug exhibits a larger proportion of patients with effective drug exposure. The main limit of this work is that we only evaluated the drug concentrations that cannot be a perfect surrogate of the drug effect. However, DOR is a promising third therapeutic agent suitable for an intermittent maintenance treatment strategy. Further studies are needed to confirm this assumption and evaluate the developed nomograms.

| Table 1 Probability of effective concentration after 72h of forgiveness based on C24h at 24h. |

502 SCREENING APPROACHES AND CLINICAL DESCRIPTION OF THE ANTICHOLINERGIC BURDEN IN PWH

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Background: Clinical impact of anticholinergic (AC) burden in people with HIV (PWH) and how to routinely detect it in clinical practice have been poorly investigated. We assessed the screening effectiveness of 3 AC scales in detecting AC symptoms and their clinical correlates in our cohort.

Methods: This cross-sectional analysis enrolled adult PWH who attended the Infectious Disease Unit of Padua Hospital (Italy) for their clinical checks (November 2021-March 2022). The AC burden scale (ABS), AC score risk (ARS), and AC drug score (ADS) were calculated. We recorded clinical data, comedications and the presence of 17 AC signs/symptoms over the last 3 month. High AC risk was defined by validated cut-offs: ABS score≥2, ARS, and ADS score≥3. Nonparametric tests and logistic regression were used; screening effectiveness of the three AC scales was assessed by the Area Under the Receiver Operating Characteristic curve (AUROC).

Results: We included 721 participants (median age 53 years, 72.0% males). Polypharmacy was present in 21.1% and 164 PWH (22.7%) were at least on 1 drug with AC properties (drug prevalence/type in Fig 1A). 4.4% PWH were classified as high AC risk by at least 1 scale (1.1%, 3.5% and 3.1% according to ARS, ABS, and ADS, respectively). Agreement between ABS and the other scales was poor (Cohen’s k 0.33 and 0.22) and moderate between ABS and ADS (k 0.60). Among PWH on AC drugs, 28.6% experienced at least 1 AC sign/symptom (prevalence/type in Fig 1B). After adjusting for univariate significant variables, factors independently associated with AC signs/symptoms were the type of antiretroviral regimen (aOR 15.2 and 32.0 for triple INSTI- and NN-based regimen vs. dual therapy, p=0.024 and 0.009; aOR 82.6 for triple protease inhibitors-based vs. dual therapy, p=0.082) and the overall number of AC drugs (aOR 9.7, p< 0.001). AUROC of ARS, ABS and ADS were 0.73 (0.63-0.82), 0.85 (0.78-0.92), and 0.84 (0.73-0.92), respectively (p< 0.001 for all). Nevertheless, at the cut-offs established for the general population the correct classification rate (78.0%, 82.9% and 84.8%) was affected by low sensitivity (34.0%, 46.8% and 46.8%).

Conclusion: AC drug use affected almost 1 out of 4 PWH in our cohort and the risk of developing AC manifestations was correlated both with the type of antiretroviral regimen and the number of drugs with AC properties. Current scales for screening AC risk in the general population showed promising use even in PWH but may require a tailored reassessment of the best cut-off.

503 PHARMACOKINETICS & SAFETY OF SUSTAINED-RELEASE FLUCYTOSINE PELLET FORMULATION

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1SFC HIV-CRYPTO Consortium

Background: WHO recommends flucytosine (SFC) as an essential component of cryptococcal meningitis (CM) treatment regimens. The currently available formulation, an immediate release (IR) tablet, needs to be administered four times a day and is sub optimal for administration via naso-gastric tube. This
study evaluated the pharmacokinetics and safety of a sustained release SFC (SR) pellet formulation suitable for twice-daily oral or naso-gastric administration in resource limited settings.

**Methods:** Phase I open-label, randomized, single dose, four-period crossover, comparative bioavailability study of SFC commercial IR tablets and SR pellets in healthy male and female participants at a single site in South Africa. The primary objective was to assess for relative bioavailability of three prototype (B, C, D) SR pellets (single dose: 1 x 3000 mg at 0 hours) relative to the reference product (A) IR Ancotil® tablets (3 x 500 mg at 0 and 6 hours after first dosing) under fasting conditions. The primary end points were plasma C<sub>max</sub> and AUC<sub>0-t</sub>. Physiologically based pharmacokinetic modelling (PBPK) was performed.

**Results:** Between Jan and April 2022, 35/42 randomised participants completed the four study treatment periods; 7 dropped out. Following administration of Ancotil (A) and SFC SR prototypes B, C and D, AUC0-t (µg*h/mL) of 471.8±72.1, 179.4±68.6, 227.8±61.2 and 258.3±64.6, and C<sub>max</sub> (µg/mL) of 40.9±8.3, 12.1±6.5, 17.3±5.8 and 20.5±5.9 for SFC, respectively were observed (Figure 1). The relative bioavailability of selected prototype D was calculated at 0.62, indicating a need for an increase in nominal dose to achieve the same exposures as Ancotil. PBPK modelling indicates that a double dose (2x3000 mg) of SFC SR prototype D is needed to achieve target SFC exposure (20-100mg/L) in fasting conditions.

No severe or serious adverse events (AEs) were reported. 36 AEs were reported in 20 participants, of which 9 (mild to moderate severity) were related to treatment. Treatment was discontinued for safety reasons in one participant in 20 participants, of which 9 (mild to moderate severity) were related to administration of Ancotil (A) and SFC SR prototypes B, C and D, AUC0-t (µg*h/mL) of 471.8±72.1, 179.4±68.6, 227.8±61.2 and 258.3±64.6, and C<sub>max</sub> (µg/mL) of 40.9±8.3, 12.1±6.5, 17.3±5.8 and 20.5±5.9 for SFC, respectively were observed (Figure 1). The relative bioavailability of selected prototype D was calculated at 0.62, indicating a need for an increase in nominal dose to achieve the same exposures as Ancotil. PBPK modelling indicates that a double dose (2x3000 mg) of SFC SR prototype D is needed to achieve target SFC exposure (20-100mg/L) in fasting conditions.

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**Conclusion:** A promising SR pellet formulation was selected for development. Single dose fasted administration of SFC in healthy participants demonstrated no new safety concerns. Further studies with the selected prototype need to be conducted with the higher dose to compare the bioavailability of IR and SR SFC in fed conditions and in people with HIV-associated CM.

Mean (SD) concentrations of S-FC by treatment in the PK population semi-log scale

**504 POPULATION PK ANALYSIS TO GUIDE DOSING WINDOW FOLLOWING LENACAVALER SC ADMINISTRATION**

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<sup>1</sup>Gilead Sciences, Inc, Foster City, CA, USA, <sup>2</sup>Centers, Inc, South Holland, Netherlands, <sup>3</sup>Centers, Inc, Sunnyvale, CA, USA

**Background:** Lenacapavir (LEN) is a potent first-in-class capsid inhibitor in development for the treatment and prevention of HIV-1 infection. Current data indicates that LEN exhibits near maximal antiviral activity when the lower bound of the 90% confidence interval (CI) of mean C<sub>min</sub> is maintained above inhibitory quotient 4 (IQ4) (at least 4-fold greater than the in vitro protein adjusted 95% effective concentration). In ongoing Phase 2/3 studies, people cannot receive SC LEN within this window, i.e., participants whose dosing falls beyond the 28-week window, restart of oral LEN loading followed by SC LEN is recommended.

**Conclusion:** In administering SC LEN every 6 months, a 4-week dosing window (+/−2 weeks around the scheduled injection) is adequate to maintain safe and efficacious exposure.

Simulated Lenacapavir C<sub>min</sub> On Week 24 to 28 in Adult People With HIV Who Received the Phase 2/3 Dosing Regimen

**Figure 1:** Simulation of Lenacapavir C<sub>min</sub> On Week 24 to 28 in Adult People With HIV Who Received the Phase 2/3 Dosing Regimen

**505 GENETICS OF HIV AND TB DRUG INTERACTIONS WITH LEVONORGESTREL EMERGENCY CONTRACEPTION**

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AIDS Clinical Trials Group A5375 Study Team

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**Background:** Levonorgestrel (LNG) is primarily metabolized by CYP3A4. Efavirenz (EFV) and rifampin (RIF) induce, while isoniazid (INH) inhibits CYP3A4. ACTG A5375, a pharmacokinetic (PK) trial of LNG emergency contraception (EC), showed that double-dose LNG (3mg vs 1.5mg) offsets the effects of EFV and RIF on LNG PK over the first 8 hours after a single dose for EC. We characterized the pharmacogenetics of these drug interactions.

**Methods:** A5375 enrolled participants in Africa, Asia, South America and the US into four groups: women living with HIV (WLH) on EFV-based ART were randomized 1:2 to LNG 1.5mg (Group A) or 3mg (Group B); WLH on dolutegravir (DTG)-based ART were assigned to 1.5mg (Group C; control group); Women treated for TB with INH/RIF were assigned to 3mg (Group D). On day 0, women received a single dose of LNG EC. Intensive PK sampling was done pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 48, 72 and 120 hours post-dose. AUC<sub>0-t</sub> was the primary outcome. Genotyping defined CYP2B6 metabolizer and NAT2 acetylator status (associated with plasma EFV and INH exposure, respectively). Linear regression models adjusted for BMI and age, and used log-transformed PK parameters.

**Results:** Of 122 cigsender women, 118 (97%) were evaluable for genetic associations: 73 (62%) identified as Black, 33 (28%) Asian, and 10 (8%) Latina. In the combined EFV Groups A/B, 8 (15%) of 52 were CYP2B6 poor metabolizers. In Group D, 15 (44%) of 34 were NAT2 slow acetylators. Within Groups A/B, CYP2B6

was utilized to simulate LEN concentrations. Various scenarios for second SC dose were simulated to evaluate the forgiveness window.
pharmacokinetics of standard vs double-dose dolutegravir after switch from efavirenz.

Rulan Griesel, Clifford Banda, Ying Zhao, Phumla Simxadi, Zaayid Omar, Graeme Meintjes, Gary Maartens
University of Cape Town, Cape Town, South Africa

Background: Dolutegravir (DTG) is the preferred second-line antiretroviral backbone agent for people living with HIV (PLWH) failing a first-line efavirenz (EFV)-based regimen. EFV induces genes associated with the metabolism and transport of DTG. The resulting drug–drug interaction between EFV and DTG significantly reduces DTG's exposure up to 14 days when switching from an EFV-based regimen. Exposure to sub-therapeutic DTG concentrations in PLWH failing EFV-based first-line ART could select for DTG resistance mutations. We conducted a pharmacokinetic study to evaluate DTG trough concentrations after switching from a failing first-line EFV-based regimen to assess the need for a lead-in supplemental dose of DTG.

Methods: We conducted a pharmacokinetic sub-study nested within a phase 2b trial (the ARTIST study) that randomised participants failing a first-line EFV-based regimen to supplemental DTG 30mg or placebo for 14 days post-switch to tenofovir/lamivudine/DTG (TLD). We obtained EFV (baseline and days 3, 7, and 14) and DTG trough plasma concentrations (days 3, 7, 14, and 28). Dose-sampling time was assessed using electronic medication dispensing devices. CYP2B6 metaboliser genotype was determined as this affects the extent of induction by EFV.

Results: We enrolled 36 participants: 11 in the placebo arm and 25 in the supplemental arm. One participant in the supplemental arm had undetectable baseline EFV and was excluded. The median age was 36 years (IQR 30–42), 77% female, baseline CD4 254 cells/mm³ (IQR 158–441), and HIV-RNA log 4.0 copies/mL (IQR 3.3–4.4). One participant in the supplemental DTG arm and none in the placebo arm had a DTG trough concentration below the PA-IC₉₀ (0.064 μg/mL) at day 3. DTG trough concentrations per arm are presented in Table 1. The difference in day 28 trough concentrations between the supplemental and placebo DTG arms was explained by CYP2B6 metaboliser genotypes: there were no slow metabolisers in the placebo arm and 5 in the supplemental arm, and DTG concentrations in normal and intermediate CYP2B6 metabolisers were similar between arms (P-values = 0.088 and 0.223, respectively).

Conclusion: No participants in the placebos arm had DTG trough concentrations below the PA-IC₉₀. Prolonged EFV induction effect in CYP2B6 slow metabolisers contributed to lower DTG trough concentrations at day 28 in the supplemental arm. Our findings do not support the need for a supplemental DTG dose when switching from a failing first-line EFV-based regimen.

Table 1. Geometric mean and geometric mean ratios of DTG trough concentration exposure when administered as standard vs double dose (N=35)

<table>
<thead>
<tr>
<th>Dosing arm</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTG standard dose (50 mg)</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>90% CI</td>
<td>(0.17 to 0.81)</td>
<td>(0.17 to 0.81)</td>
<td>(0.17 to 0.81)</td>
<td>(0.17 to 0.81)</td>
</tr>
<tr>
<td>DTG double dose (100 mg)</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>90% CI</td>
<td>(0.13 to 0.53)</td>
<td>(0.13 to 0.53)</td>
<td>(0.13 to 0.53)</td>
<td>(0.13 to 0.53)</td>
</tr>
</tbody>
</table>

* P-value < 0.05
** P-value < 0.01
508 INTRAMUSCULAR CABOTEGRAVIR/RILPIVIRINE CONCENTRATIONS AFTER SWITCHING FROM EFAVIRENZ
Sara Bettonte1, Mattia Berton1, Felix Stader2, Manuel Battegay3, Catia Marzolini1
1University Hospital Basel, Basel, Switzerland, 2Certara, Inc, Sheffield, United Kingdom, 3University Hospital Basel, Basel, Switzerland.

Background: Intramuscular cabotegravir and rilpivirine (IM CAB/RPV) are used once viral load suppression is achieved on another antiretroviral regimen. While CAB/RPV are substrates of UGT1A1/CYP3A4, efavirenz induces these enzymes therefore switching from an efavirenz containing regimen to IM CAB/RPV could possibly result in a time window with suboptimal drug levels. The aim of this study was to simulate the initial IM CAB/RPV concentrations after stopping efavirenz using physiologically based pharmacokinetic (PBPK) modelling.

Methods: The in-house PBPK model implemented with a mechanistic intramuscular framework was validated against observed clinical data. Prior to simulating IM CAB/RPV concentrations after switching from efavirenz, we firstly verified the models for CAB, RPV, and efavirenz separately. Secondly, we simulated the switch from efavirenz to oral RPV and dolutegravir (another UGT1A1 substrate). The model was considered validated when the predictions were within 2-fold of clinical data. A cohort of 100 virtual individuals (20-50 years old, 50% female, 18-30 kg/m²) was generated to simulate IM CAB/RPV concentrations over time when administering CAB/RPV (600/900 mg) 12 hours after the last oral dose of efavirenz (600 mg). IM CAB/RPV concentrations during the switch period were compared to those in absence of residual efavirenz concentrations.

Results: The model was successfully verified as all predictions were within 2-fold of observed clinical data. Initiating IM CAB/RPV 12 hours after the last dose of efavirenz was predicted to have a minimal effect as IM CAB concentrations (C) were reduced by 11%, 13% and 8% at days 1, 7 and 14 after discontinuing efavirenz (Table 1). For all time points, CAB C was above the 4-fold PA-IC90. Similarly, efavirenz was predicted to have a modest effect on IM RPV concentrations with the lowest reduction being 10% and occurring 7 days after the last dose of efavirenz. Residual efavirenz concentrations were predicted to have a less pronounced effect on IM RPV compared to the observed switch data with oral RPV (e.g., IM RPV reduced by 8% vs 28% for oral RPV at day 14 post efavirenz) (Capues et al. Antiviral Therapy 2012).

Conclusion: The PBPK model demonstrates that switching from an efavirenz-containing regimen to IM CAB/RPV does not put at risk of having a time window with suboptimal drug levels.

Table 1: Predictions of IM cabotegravir and rilpivirine concentrations at days 1, 7, 14, and 28 after stopping efavirenz.

<table>
<thead>
<tr>
<th>CAB</th>
<th>Cg</th>
<th>CAB</th>
<th>Cg</th>
<th>CAB</th>
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<tr>
<td>IM CAB</td>
<td>C</td>
<td>IM CAB</td>
<td>C</td>
<td>IM CAB</td>
<td>C</td>
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<tr>
<td>IM</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Day 1</td>
<td>421 (20)</td>
<td>373 (20)</td>
<td>0.89</td>
<td>1202 (46)</td>
<td>1192 (46)</td>
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<tr>
<td>Day 14</td>
<td>134 (5)</td>
<td>105 (5)</td>
<td>0.93</td>
<td>986 (58)</td>
<td>930 (54)</td>
</tr>
<tr>
<td>Day 28</td>
<td>45 (17)</td>
<td>42 (16)</td>
<td>0.94</td>
<td>79 (24)</td>
<td>71 (21)</td>
</tr>
<tr>
<td>Day 14</td>
<td>45 (36)</td>
<td>41 (29)</td>
<td>0.92</td>
<td>31 (20)</td>
<td>30 (20)</td>
</tr>
</tbody>
</table>

509 SWITCHING OFF EFAVIRENZ TO NON-EFAVIRENZ ART LOWERS NICOTINE METABOLITE RATIO IN PWH
Robert Gross1, Warren Bilker2, Xiaoyan Han3, Jessica Merlin1, Michael Plankey4, Jeffrey Martin2, Heidi Crane1, Leila Hojat1, Laura Bamford7, Robert Schnoll1, Matthew Rizk1, Julie Stone1
1Mercy & Co Inc, West Point, PA, USA, 2Mercy & Co Inc, Rathway, NJ, USA.

Background: Cigarette smoking in people with HIV (PWH) is 2-3x that of people without HIV, over 40% in some US groups, and causes more morbidity than HIV itself in smokers with viral control. Nicotine is metabolized by CYP2A6, measured by nicotine metabolite ratio (NMR; 3-hydroxy cotinine/cotinine) and higher NMR is associated with lower quit rates. In trials, PWH have lower quit rates than expected from studies in people without HIV. Efavirenz, still a mainstay antiretroviral therapy (ART) drug in lower income countries, upregulates its own metabolism. We hypothesized that efavirenz increases NMR compared with non-efavirenz ART.

Methods: We compared NMR from plasma during and post-efavirenz use among current cigarette smokers with HIV in the CNICS cohort. Eligibility included having stored plasma at two time points when smoking cigarettes and HIV RNA < 400 copies/ml (1) on efavirenz and (2) after switching to non-efavirenz ART. Cotinine and 3-hydroxycotinine were measured in plasma using liquid chromatography-tandem mass spectrometry. We used signed rank tests to compare NMR on and off efavirenz. We targeted enrolling at least 71 pairs for 80% power to detect a clinically meaningful 0.1 unit increase in NMR with p<0.05.

Results: We analyzed samples between January 2010 and November 2019 in 72 PWH, 63 (88%) men, 34 (47%) Black, median age 51 years (range: 21-66) with 53 (74%) switching to integrase inhibitor-based and 19 (26%) switching to non-nucleoside analog based ART. Specimens were a median of 629 days apart (range 133, 3157 days) with median plasma cotinine on efavirenz of 164 ng/ml (IQR: 128, 218) and median plasma cotinine of 180 ng/ml (IQR: 138, 332) on non-efavirenz ART. The median NMR on efavirenz was 0.73 (IQR: 0.52, 0.98) and on non-efavirenz ART was 0.46 (IQR: 0.29, 0.67) with a median difference of 0.26 decrease (IQR: -0.41, -0.007, p< 0.001) with 44 (61%) having NMR decrease by at least 0.1. The findings were not changed when stratified by age, race, sex assigned at birth, or non-efavirenz ART regimen type.

Conclusion: Switching off efavirenz resulted in substantial decreases in the NMR. Our findings suggest that previously observed higher NMR among PWH may be due to direct pharmacologic effects of individual ART drugs. If subsequent studies show higher quit rates in those on non-efavirenz than efavirenz-based ART, assessing each ART drug’s effect on NMR may inform ART choice in HIV+ smokers away from nicotine metabolism inducers to potentially increase smoking cessation rates in PWH.

510 IMMUNE-VIRAL DYNAMICS MODELING OF THE BASIS FOR INDIVIDUAL VARIATION IN COVID-19
Youfang Cao1, Akshita Chawala1, Brian Maas1, Ruthie Birger1, Laura Liao1, Matthew Rizk1, Julie Stone1
1Mercy & Co Inc, West Point, PA, USA, 2Mercy & Co Inc, Rathway, NJ, USA.

Background: Viral dynamics models provide mechanistic insights into viral disease and therapeutic interventions. A detailed, mechanistic model of COVID-19 was developed and fit to data from molnupiravir (MOV) trials to characterize the SARS-CoV-2 viral dynamics in MOV-treated and untreated participants and describe the basis for variation across individuals.

Methods: An Immune-Viral Dynamics Model (IVDM) incorporating mechanisms of viral infection, viral replication, and innate and adaptive immune response described the dynamics of viral load (VL) from pooled data from MOV Phase 2 and 3 trials (N=1958). Population approaches were incorporated to estimate variation across individuals and to conduct an extensive covariate analysis. Nineteen parameters in a system of five differential equations described SARS-CoV-2 viral dynamics in humans. Six population parameters were successfully informed through fitting to observed trial data while the remaining parameters were fixed based on literature values or calibrated via sensitivity analysis.

Results: Final viral dynamics and immune response parameters were all estimated with high certainty and reasonable inter-individual variabilities. The model captured the viral load profiles across a wide range of subpopulations and predicted lymphocyte dynamics without using this data to inform the parameters, suggesting inferred immune response curves from this model were accurate. This mechanistic representation of COVID-19 disease indicated that the processes of cellular infection, viral production, and immune response are in a time-varying, non-equilibrium state throughout the course of infection. MOV mechanism of action was best described as an inhibitory process on the infectivity term with estimated AUC50 of 10.5 μM*hr. Covariates identified included baseline viral load on infectivity and age, baseline disease severity, viral clade, baseline viral load, and diabetes on immune response parameters. Greater variation was identified for immune parameters than viral kinetic parameters.

Conclusion: These findings show that the variation in the human response (e.g., immune response) is more influential in COVID-19 disease than variations
in the virus kinetics. The model indicates that immunocompromised patients (due to HIV, organ transplant, active cancer, immunosuppressive therapies) develop an immune response to SARS-CoV-2, albeit more slowly than in immunocompetent, and MOV is effective in further reducing viral loads in the immunocompromised.

511 FACTORS INFLUENCING COVID-19 RISK: INSIGHTS FROM MOLNUPIRAVIR EXPOSURE-RESPONSE
Akshita Chawla1, Brian Maas1, Ruthie Birger1, Hong Wan1, Youfang Cao2, Matthew Johnson1, Carisa De Anda1, Matthew Rizk1, Julie Stone1
1Merck & Co, Inc, Rathway, NJ, USA, 2Merck & Co, Inc, West Point, PA, USA, 3Merck & Co, Inc, North Wales, PA, USA

Background: Exposure-response (E-R) models were developed for the primary endpoint of hospitalization or death in COVID-19 patients from the Phase 3 portion of the MOVe-OUT study (Clinicaltrials.gov NCT04577797). Beyond dose, these models can identify other determinants of response, highlight the relationship of virologic response with clinical outcomes, and provide a basis for differential efficacy across trials.

Methods: Logistic regression models were constructed using a multi-step approach with influential covariates identified first using placebo arm data only. Subsequently the assessment of drug effect based on drug exposure was determined using placebo and molnupiravir (MOV) arm data. To validate the models, the rate of hospitalization/death was predicted for published studies of COVID-19 treatment. All work was performed using R Version 3.0.0 or later.

Results: A total of 1313 participants were included in the E-R analysis, including subjects having received MOV (N=630) and placebo (N=683). Participants with missing baseline RNA or PK were excluded (79 from MOV and 16 from placebo arms). The covariates shown to be significant determinants of response were baseline viral load, baseline disease severity, age, weight, viral clade, and co-morbidities of active cancer and diabetes. Day 5 and Day 10 viral load were identified as strong on-treatment predictors of hospitalization/death, pointing to sustained high viral load as driving negative outcomes. Estimated AUC50 was 19900 nM*hr with bootstrapmed 95% C.I. of (9270, 32700). In an external validation exercise based on baseline characteristics, the E-R model predicted the mean (95% CI) placebo hospitalization rates across trials of 9.3% (7.6%, 11.7%) for MOVe-OUT, 7.2% (5.3%, 9.8%) for the nirmatrelvir/ritonavir EPIC-HR trial, and 3.2% (1.9%, 5.5%) for generic MOV trials by Aurobindo and Hetero, consistent with the differing observed placebo rates in these trials. The relative reduction in hospitalization/death rate predicted with MOV treatment (relative to placebo) also varied with the above patient populations.

Conclusion: Overall, the exposure-response results support the MOV dose of 800 mg Q12H for treatment of COVID-19. The results further support that many clinical characteristics impacted hospitalization rate beyond drug exposures which can vary widely across studies. These characteristics also influenced the magnitude of relative risk reduction achieved by MOV in the MOVe-OUT study.

512 NO DOSE ADJUSTMENTS FOR CYP3A4 SUBSTRATES WHEN COADMINISTERED WITH BEMNIFOSBUVIR
Xiao-Jian Zhou1, Gaetano Morelli1, Maureen Montondo1, Keith Pietropaolo1, Bruce Belanger1, Arantxa Horga1, Janet Hammond1
1Arena Pharmaceuticals, Inc., Boston, MA, USA, 2Altasciences, Laval, QC, Canada

Background: Bemnifosbuvir (BEM, AT-527) is a guanosine nucleotide prodrug candidate for the treatment of COVID-19 and chronic HCV. BEM was identified in vitro as an inhibitor of drug transporters P-glycoprotein, breast cancer resistant protein (BCRP) and organic anion transporting polypeptide 1B1 (OATP1B1). Ph1 studies in healthy participants were conducted to assess the clinical implications of these results using digoxin (DIG) and rosuvastatin (ROSU) as P-gp and BCRP/OATP1B1 index drugs, respectively.

Methods: Both studies employed a similar design with 2 groups of 14 healthy participants: Day 1/period 1, all participants received a single dose of DIG 0.25mg or ROSU 10mg alone. In period 2, participants received DIG 0.25mg or ROSU 10mg with BEM 1100mg, simultaneously (n=14) or staggered by 2h (n=14). Serial plasma samples were collected and quantitated for DIG or ROSU concentrations.

Results: A single dose of BEM 1100mg simultaneously administered slightly increased the Cmax of DIG (178%), yet had no effect on its AUC, consistent with the transient nature of BEM plasma PK. When dosed staggered, BEM did not affect the PK of DIG. A single dose (simultaneous or staggered) of BEM 1100mg slightly increased the plasma exposure of ROSU (20%–40%). There was no effect on vital signs, ECG, and no SAEs or drug discontinuations.

Conclusion: A single high dose of BEM 1100mg only slightly increased the plasma exposure of the P-gp and BCRP/OATP1B1 index drugs DIG and ROSU. BEM has low potential to exhibit clinical meaningful inhibition of these transporters. No dose adjustment will be needed for drugs that are sensitive substrates of P-gp or BCRP/OATP1B1 when co-administered with BEM, staggered dosing may lessen any DDI risk.

513 BEMNIFOSBUVIR HAS LOW POTENTIAL TO INHIBIT P-gp, BCRP, AND OATP1B1 MEDIATED TRANSPORT
Xiao-Jian Zhou1, Gaetano Morelli1, Maureen Montondo1, Keith Pietropaolo1, Bruce Belanger1, Arantxa Horga1, Janet Hammond1
1Arena Pharmaceuticals, Inc., Boston, MA, USA, 2Altasciences, Laval, QC, Canada

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Conclusion: A single high dose of BEM 1100mg only slightly increased the plasma exposure of the P-gp and BCRP/OATP1B1 index drugs DIG and ROSU. BEM has low potential to exhibit clinical meaningful inhibition of these transporters. No dose adjustment will be needed for drugs that are sensitive substrates of P-gp or BCRP/OATP1B1 when co-administered with BEM, staggered dosing may lessen any DDI risk.

514 PHARMACOKINETICS INFORMS REMDESIVIR DOSING FOR PATIENTS WITH SEVERE RENAL IMPAIRMENT
Rita Humeniuk1, Hong Deng1, Inmaculada C. Sorribes2, Sean Regan3, Haeyoung Zhang4, Richard Robinson5, Megan Site6, Yannis Kouillas7, Joe Llewellyn8, Robert Hyland9, Anu Osimus10, Sandhya Girish11, Ana Ruiz-Garcia11, Helen Winter11
1Gilead Sciences, Inc, Foster City, CA, USA, 2Ventara, Inc Durham, NC, USA, 3New Zealand Clinical Research, Christchurch, New Zealand, 4Massachusetts General Hospital of Renal Associates, Boston, MA, USA

Background: Despite renal impairment (RI) being a risk factor for severe COVID-19, there are no approved antiviral treatment options for patients with severely impaired kidney function (eGFR less than 30 mL/min/1.73 m2 or kidney failure) in the US. At the time remdesivir (RDV) was initially approved for the treatment of COVID-19, the impact of renal impairment (RI) on pharmacokinetics (PK) of RDV, its metabolites, and the excipient, sulfobutylether β-cyclodextrin sodium (SBECD), was not known.

Methods: Here, we report the PK data supporting dosing of RDV in COVID-19 patients with severely impaired kidney function. PK samples for RDV and metabolites (GS-704277, GS-441524) were collected in the Phase 3 REDPINE study in hospitalized COVID-19 patients with severely impaired kidney function. Participants in this double-blind study were randomized 2:1 to intravenous (IV) remdesivir (200 mg on Day 1, then 100 mg daily up to Day 5) or IV saline as placebo-to-match. SBECD PK was analyzed in a phase 1 study in non-COVID-19 patients with normal kidney function, mild and moderate RI who received 100 mg dose of remdesivir (containing 3000 mg SBECD). The population PK analysis included observations from healthy and COVID-19 patients with full range of renal function across all adult studies.

Results: Geometric mean exposures (AUCinf) observed in REDPINE Study as compared to PINETREE Study increased up to 553% for the GS-441524 metabolite (dependent on renal elimination) and to a lesser degree GS-704277 (294%, minor renal elimination) and RDV (78.9%; an increase explained by factors other than renal function, namely, hospitalization and body weight)
Conclusion: Significant efforts have been made by the 3 teams to provide up-to-date, complementary DDI guidance. Usage metrics confirm the demand for DDI guidance during the pandemic. Cross-utilization of the DDI guides confirms the need for consistency. DDI recommendations were more permissive than initial product information, expanding clinicians' ability to prescribe NMV/r. DDI guidance for ACs and immunosuppressants was particularly challenging. During drug development, complex interactions likely to be encountered in target populations should be addressed.

Table 1. Description and 2022 Metrics for NMV/r DDI Guides

<table>
<thead>
<tr>
<th>Description</th>
<th>University of Liverpool</th>
<th>NIH Guidelines</th>
<th>Ontario Science Table</th>
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<tbody>
<tr>
<td>DDI guide</td>
<td>Web-based, interactive DDI checker</td>
<td>Webpage/pdf</td>
<td>Webpage/pdf (DDI table and DDAC algorithm)</td>
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</table>

<table>
<thead>
<tr>
<th>No. of drugs in DDI guide</th>
<th>No. of DDI guides</th>
<th>No. of drugs included in DDI guide</th>
<th>No. of drugs in prior version</th>
<th>No. of users logging into DDI checker</th>
<th>No. of requests to drug entries</th>
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<tbody>
<tr>
<td>Total</td>
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<td>12,000</td>
<td>12,000</td>
<td>143</td>
<td>40</td>
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<tr>
<td>NMV/r</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>125</td>
<td>20</td>
</tr>
<tr>
<td>NIRMATRELVIR/RITONAVIR</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>120</td>
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</table>

515 GLOBAL COLLABORATION TO PROVIDE DRUG INTERACTION GUIDANCE FOR NIRMATRELVIR/RITONAVIR

Safia Kuriakose1, Alice Tseng2, Sara Gibbons1, Fionna Marra1, Alison Boyle1, Tessa Saye Kenney3, Gregory Eschenauer4,5, Sarita Boyd6, Jomy George7, Kimberly K. Scarsi8,9, and Stephanie Kenney10

Background: Nirmatrelvir/ritonavir (NMV/r), a preferred antiviral for high-risk outpatients with COVID-19, is associated with major drug-drug interactions (DDIs). Given the lack of DDI data with short course ritonavir (RTV), initial NMV/r product information was extrapolated from chronic, full dose RTV use. In Jan 2022, DDI experts from the University of Liverpool (UoL), NIH COVID-19 Guidelines Panel, and Ontario Science Table (OST) contributors established a global collaboration to address DDI challenges limiting NMV/r use in real-life settings. We report how safe, pragmatic, and consistent resources were developed to support NMV/r prescribing, and the utilization of these resources globally.

Methods: The 3 teams met monthly to discuss DDIs, review NMV/r DDI literature, and achieve consensus on recommendations. Additional experts were invited as needed. Metrics from the UoL DDI checker guided review of most searched DDIs overall and by severity. 2022 usage metrics for each DDI guide were extracted from the electronic medical record. Outcomes of interest were comparison of pre- and on-treatment HIV RNA values, and at least one on-treatment HIV RNA value ≥20 copies/mL (c/mL). Data are lacking evaluating predictors of varying levels of on-treatment elevations in viral load in patients receiving long-acting cabotegravir plus rilpivirine (CAB/RPV) for people with HIV (PHW). Study objectives were to I) quantify the occurrence of detectable viremia on CAB/RPV and II) determine if HIV RNA values in the preceding year predicted virologic outcomes.

Results: A retrospective cohort study was performed among those receiving care at the University of California San Diego Health Center. Inclusion criteria involved treatment with CAB/RPV for at least three months, availability of both pre- and on-treatment HIV RNA values, and at least one on-treatment HIV RNA value ≥20 c/mL. Data were extracted from the electronic medical record. Outcomes of interest were incidence of on-treatment HIV RNA values ≥20 c/mL, ≥50 c/mL, and ≥200 c/mL.

Conclusions: Despite virologic suppression at time of switch to CAB/RPV, over 25% of patients experienced at least one HIV RNA ≥20 c/mL with incidence highest among patients with at least one HIV RNA ≥20 c/mL in the preceding year. While the impact of low-level increases in viral load is not entirely clear,
Projecting Benefits of Long-Acting ART in PWH with Viremia Despite Prescribed Oral ART

Wanyi Chen, Monica Gandhi, Paul E. Sax, Anne Neilan, Justine Scott, Timothy Wilkin, Andrea L. Ciaramello, Joseph J. Eron, Kenneth A. Freedberg, Emily P. Hyle

Background: Long-acting injectable antiretroviral therapy (LA-ART) with cabotegravir-rilpivirine (CAB-RPV) is approved for PWH with viral suppression on oral ART. A recent pilot project showed that monthly CAB-RPV with wraparound services was effective for viral suppression in most patients, but remained viremic despite prescribed oral ART. We projected the clinical impact of providing LA-ART with wraparound social services (WS) compared with oral ART with or without WS for patients who had viremia despite oral ART. A recent pilot project showed that monthly CAB-RPV with wraparound services (WS) resulted in viral suppression in most patients with viremia despite prescribed oral ART. Projected clinical benefits were greater in patients with lower CD4 counts.

Methods: Using the CEPAC microsimulation model, we compared 3 strategies: 1) Standard of care using oral INSTI-based ART (SOC), 2) SOC with WS (SOC/WS), and 3) LA-ART with WS (LA-ART/WS). WS consisted of community-based supports (e.g., case managers, home- and street-based nursing services). Model outcomes included viral suppression, changes in CD4, retention in care at 2y, life expectancy (LYs), and transitions to PI-based regimens for failure with INSTI resistance. Base case cohort characteristics were based on published data: mean age 40y; 87% male; mean (SD) CD4 100 (50)/µl. Viral suppression at 6m was 25% (SOC) and 49% (SOC/WS) from published data, and 72% (LA-ART/WS); assumption. Mean (SD) loss to follow-up rates were 29.0 (10.3)/100PY, and 49% (SOC/WS); and 72% (LA-ART/WS). In sensitivity analysis, we varied all key parameters.

Results: Viral suppression at 2y varied widely: 22% SOC, 45% SOC/WS, and 63% LA-ART/WS (Table). Projected life expectancy was 10.22 LY SOC, 13.53 LY SOC/WS, and 17.09 LY LA-ART/WS. Of those initiating LA-ART, 58% experienced virologic failure on LA-ART over their lifetime and transitioned to a PI-based regimen. Projected clinical benefits were greater with LA-ART and lower CD4 counts but remained substantial in PWH with higher CD4 counts. For PWH with lower ART adherence and engagement in care, LA-ART/WS still resulted in substantial gains in life expectancy (+5.4y compared with SOC/WS). Even if LA-ART efficacy was as low as 20% at 3 months and WS improved care retention by only 5% at 2y, LA-ART/WS would still result in greater viral suppression and major increases in life expectancy (+1.4y compared with SOC/WS).

Conclusion: For PWH with persistent viremia despite prescribed oral ART, LA-ART with CAB-RPV and wraparound services is likely to substantially increase viral suppression and improve survival for this difficult-to-treat population. A clinical trial to provide further supportive evidence is urgently needed.
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THIGH INJECTIONS OF CABOTEGRAVIR + RILPIVIRINE IN VIRALLY
SUPPRESSED ADULTS WITH HIV-1

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Background: Cabotegravir (CAB) + rilpivirine (RPV) long-acting (LA) administration via gluteal intramuscular (IM) injections is recommended by treatment guidelines for maintaining HIV-1 virologic suppression. Previous data in healthy participants receiving single CAB + RPV LA IM injections to the vastus lateralis (thigh muscle) were supportive of further evaluation. Here we present the results of a substudy evaluating the pharmacokinetics (PK), safety, tolerability, and efficacy of CAB + RPV LA following short-term repeat IM thigh administration in adults with HIV-1 who had received ≥3 years of gluteal injections while participating in the ongoing Phase 3b ATLAS 2M study.

Methods: ATLAS-2M participants volunteered and reconsented for the substudy, which included a screening phase, thigh injection phase (Day 1–Week 16), and return to gluteal injection phase (W16–24). The substudy injection schedule was unchanged from the main study (every 8 week arm (Q8W) arm, CAB 600 mg + RPV 900 mg; every 4 week (Q4W) arm, CAB 400 mg + RPV 600 mg). CAB and RPV PK parameters following the last thigh gluteal injections and first and last thigh injections were determined by noncompartmental analysis and compared by mixed-effects modeling. Safety, tolerability, participant-reported outcomes, and efficacy were assessed.

Results: 118 participants (Q8W, n=54; Q4W, n=64) enrolled; median (range) age was 48 years (24–71), 38% were female sex at birth, 82% were White. In the Q8W arm, first CAB thigh injection AUC (0–t) and Cmax, first RPV thigh injection AUC (0–t) and Cmax were similar to gluteal administration, with no clinically significant differences observed. These results support rotational/short-term CAB + RPV LA IM lateral thigh administration within an established gluteal regimen. Additional analyses will assess the potential for early or chronic thigh administration. Table. Geometric Least Squares Mean Ratios and 90% Confidence Intervals for CAB and RPV Parameters Following Thigh (Test) and Gluteal (Reference) Administration by Injection Interval and Treatment Arm (Paired Data)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameter</th>
<th>Q8W Mean</th>
<th>Q8W</th>
<th>Q4W Mean</th>
<th>Q4W</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB</td>
<td>Cmax</td>
<td>1.35 (1.22, 1.48)</td>
<td>1.35 (1.09, 1.62)</td>
<td>1.35 (1.09, 1.62)</td>
<td>1.35 (1.09, 1.62)</td>
</tr>
<tr>
<td></td>
<td>AUC(0-t)</td>
<td>0.90 (0.87, 0.93)</td>
<td>0.90 (0.87, 0.93)</td>
<td>0.90 (0.87, 0.93)</td>
<td>0.90 (0.87, 0.93)</td>
</tr>
<tr>
<td>RPV</td>
<td>Cmax</td>
<td>1.29 (1.23, 1.35)</td>
<td>1.29 (1.23, 1.35)</td>
<td>1.29 (1.23, 1.35)</td>
<td>1.29 (1.23, 1.35)</td>
</tr>
<tr>
<td></td>
<td>AUC(0-t)</td>
<td>1.08 (1.05, 1.11)</td>
<td>1.08 (1.05, 1.11)</td>
<td>1.08 (1.05, 1.11)</td>
<td>1.08 (1.05, 1.11)</td>
</tr>
</tbody>
</table>

Conclusion: CAB and RPV parameters following 16 weeks of thigh injections were similar to gluteal administration, with no clinically significant differences observed. These results support rotational/short-term CAB + RPV LA IM lateral thigh administration within an established gluteal regimen. Additional analyses will assess the potential for early or chronic thigh administration.

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IMPACT OF BASELINE FACTORS ON VIROLOGIC RESPONSE TO bNAb VH3810109 IN BANNER

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Background: Broadly neutralizing antibodies (bNAbs) are being developed for long-acting HIV-1 therapy. VH3810109 (N6LS) is a CD4-b antibody with broad and potent neutralization activity in vitro, which is currently being evaluated in the Phase 2a BANNER study.

Methods: BANNER is a randomized, open-label, 2-part, multicenter study in treatment-naïve viremic (viral load [VL] ≥5000 c/mL) adults to evaluate safety, pharmacokinetics, and antiviral activity of VH3810109. In part 1, VH3810109 was evaluated during monotherapy after a single IV infusion of 40mg/kg or 280mg (~4mg/kg) followed by 48 weeks of standard-of-care ART. Monotherapy duration was determined by either virologic non-response (VL < 0.5 log10, by Day 11) or rebound (VL ≥1.0 log10 over nadir or < 0.5 log10, from baseline [BL]). Antibody sensitivity of pre-dose and rebound viruses was determined retrospectively, using the PhenoSense mAb assay. Here we report the impact of BL viral and participant factors, on maximum VL decline (VLD) and time to virologic rebound following infusion of VH3810109.

Results: Fourteen participants enrolled, 13 were male, median (range) BL VL was 4.31 (3.13-5.24) log10 c/mL and median (range) BL CD4+ count was 369 (190-700) cells/mm3. Virologic response was observed in 13 participants; median (range) viral nadir from BL was 1.72 (0.60-2.60) and 1.18 (0.30-2.18) log10 c/mL for 40mg/kg and 280mg, respectively. In a post hoc analysis, BL VH3810109 IC50 and CD4+ cell count were moderately correlated with maximum VLD and time to viral rebound in both treatment arms. The 2 participants with the highest IC50 (1.512 and >350 µg/mL) had the smallest VLD (0.60 and 0.30 log10 c/mL) and the shortest time to rebound (12 days and virologic non-response). The longest time to rebound (78 days) was observed in the participant with the lowest IC50 (0.091 µg/mL). A weak correlation between lower BL log10 HIV-1 RNA and virologic response was apparent only in the 280mg dose group.

Conclusion: In BANNER part 1, a single IV infusion of VH3810109 was well tolerated, with few drug-related AEs and robust antiviral efficacy at both doses studied. In this small study, viral sensitivity to VH3810109 and BL CD4+ cell count correlated with magnitude and duration of antiviral response. However, other factors may impact virologic outcome, including pre-treatment VL, serum antibody concentration, and an individual’s inherent control of viral replication. BANNER part 2 is ongoing to evaluate alternate dosing options and modalities for VH3810109.

Figure. Viral Responses and Baseline Antibody Sensitivity of Plasma Viruses

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameter</th>
<th>Q8W Mean</th>
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<tr>
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<td>AUC(0-t)</td>
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</tr>
</tbody>
</table>

*40 µg/kg does assume a body weight of ~72 kg. IC50 values of 50 µg/mL represents the highest concentration tested in the PhenoSense mAb assay.
Rapid ART initiation using BIC/FTC/TAF and TDF+3TC+EFV in People with HIV in China

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Background: The benefits of antiretroviral therapy (rapid ART) has been widely proven among people with HIV, but related data are still limited in China. This study examined virological outcomes and the treatment retention rate at 24 weeks after rapid versus non-rapid ART initiation, and analyzed the efficacy of EFV (EFV+3TC+TDF) and Bictegravir 50 mg/Emtricitabine 200 mg/Tenofovir Alafenamide 25 mg (BIC/FTC/TAF) for rapid ART.

Methods: This was a national, open label, pragmatic randomized controlled trial. We enrolled all the HIV-1 infected adult (age ≥ 18 years) men who have sex with men (MSM) diagnosed from March 2021 to April 2022 across eight sites in China. The participants chose to start ART within 14 days after HIV diagnosis were randomly assigned (1:1) to the EFV group (A) and BIC group (B); those who did not need rapid ART used EFV (C) or BIC (D) voluntarily. The primary endpoint was the percentage of viral suppression (< 50 copies/ml) after 24 weeks.

Results: A total of 495 participants were enrolled, including 126, 132, 122 and 91 participants in A, B, C, D group respectively. In the rapid (group A and B) and non-rapid ART (group C and D) groups, 92.6% and 96.9% (P = 0.053) participants retained in care (Figure 1). Viral suppression rate was higher in group B than in group A (93.5% vs. 74.2%, P < 0.001) but similar between group A and group C (74.3% vs. 76.1%, P = 0.16) for EFV-efavirenz. In group B, 33.3% patients changed from underweight (BMI < 18.5) at baseline to normal weight (BMI ≥ 18.5) by 24 weeks after ART initiation, and 8.1% patients in group B changed from overweight to obese (BMI ≥ 25) after 24 weeks. 10% patients and 18.0% patients changed from normal weight to overweight (BMI ≥ 25; BMI < 30) in group A and B, 5.3% patient in group A and 8.1% patients in group B changed from overweight to obese (BMI ≥ 30) respectively. Total serum cholesterol levels increased in both groups (+0.03 VS +0.47 mmol/L, P = 0.001). The level of LDL was reduced in group A, while increased in group B after 24 weeks compared to baseline (-0.22 VS +0.27 mmol/L, P < 0.001). Changes of HDL (+0.10 VS +0.47 mmol/L, P = 0.001) and triglycerides (+0.04 VS +0.09 mmol/L, P = 0.88) were not statistically significant.

Conclusion: Rapid ART was associated with a good retention rate of care, BIC/FTC/TAF was effective for rapid ART.

Long-acting Lenacapavir in a Combination Regimen for Treatment-naive HIV-1 in China: A randomized controlled trial

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Background: Lenacapavir (LEN) is a potent first-in-class long-acting HIV-1 capsid inhibitor, is in development for treatment and prevention of HIV-1. CALIBRATE is an ongoing Phase 2 open-label active-controlled study evaluating subcutaneous (SC) and oral LEN in combination with other antiretrovirals, in people with HIV-1 (PWH) who are initiating treatment. At the Week 54 (W54) primary endpoint, SC LEN every 6 months or oral LEN daily (QD) in combination with tenofovir alafenamide (TAF), bictegravir (BIC), or emtricitabine (FTC) /TAF, maintained high rates of virologic suppression (90%, 85%, 85%, respectively) and was generally well tolerated.

Methods: Participants were randomized (2:2:2:1) to 1 of 4 treatment groups (TG). TG1 and TG2 both received SC LEN + oral QD FTC/BIC for 28 weeks, after which virologically suppressed participants continued a 2-drug maintenance regimen: SC LEN with QD TAM (TG1) or QD BIC (TG2). TG3 received oral QD LEN + FTC/TAF, and TG4 received oral QD FTC/BIC/TAF throughout. We report the secondary efficacy endpoint by FDA Snapshot algorithm and safety at W80. The study did not have prespecified formal statistical comparisons between TGs.

Results: 182 participants (7% female, 52% Black) were randomized and dosed (n = 52, 53, 52, 25 in TG1 to TG4). Median age was 29 years; 15% had viral load (VL) >100,000 c/mL. At W28, 94%, 92%, 94%, and 100% had VL < 50 c/mL. At W80, 87%, 76%, 87%, and 92% had VL < 50 c/mL; most remaining participants had discontinued study drug for reasons other than efficacy or safety (e.g. loss to follow up, participant decision, investigator decision: TG1 [n = 6, 11%], TG2 [n = 9, 17%], TG3 [n = 6, 11%]). By missing-exclusion analysis, 98%, 100%, 98% and 96% had VL < 50 c/mL. For participants in TG1 to TG3, CD4 count increased by a median of 249 cells/µL (IQR 122, 384) at W80 (223 [92, 350] in TG4). No participant experienced a study drug-related serious adverse event (SAE). No participants discontinued LEN due to AE's after W54. In TG1 to TG3, the most frequent non-injection site reaction (ISR) AE's were headache (16%) and nausea (13%). LEN-related ISRs were mostly mild to moderate.

Conclusion: LEN, given SC or orally in combination with TAF, BIC, or FTC/TAF, maintained high rates of virologic suppression through W80 and was well tolerated. These results support ongoing evaluation and further development of LEN in combination with other long-acting partner agents for the treatment of HIV-1 infection and support Gilead's long-acting oral and injectable development program.
Day 15 (D15), those on oral LEN received subcutaneous (SC) LEN 927 mg every 6 months (Q6M); those on placebo started the oral lead-in, followed by SC Q6M. Randomized participants discontinued the failing regimen and initiated an investigator-selected OBR at D15. In the nonrandomized cohort, participants initiated OBR concurrent with LEN (OBR concurrent with LEN oral lead-in). Week 52 efficacy was assessed in both cohorts using FDA snapshot algorithm.

**Results:** 72 participants enrolled (36 in each cohort). 46% (33/72) had 4-class resistance (NRTI, NNRTI, PI, and INSTI); 17% (12/72) had no fully active agents in the OBR. High rates of virologic suppression were achieved among participants who had baseline high HIV-1 RNA, low CD4, INSTI resistance, suboptimal OBR, and regardless of use of DTG and DRV in the OBR (Table).

**Conclusion:** In this population of PWH who were heavily treatment-experienced with limited treatment options due to MDR HIV, LEN in combination with OBR led to high rates of virologic suppression, regardless of baseline HIV-1 RNA, CD4 count, INSTI resistance, and number of fully active OBR agents.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Randomized and nonrandomized volumes (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>78% (58/72)</td>
</tr>
<tr>
<td>Baseline CD4 &lt; 200 cells/μL</td>
<td>73% (29/40)</td>
</tr>
<tr>
<td>Baseline HIV-1 RNA, &gt;100,000 copies/mL</td>
<td>88% (37/42)</td>
</tr>
<tr>
<td>Baseline INSTI resistance</td>
<td>78% (57/73)</td>
</tr>
<tr>
<td>Without INSTI resistance</td>
<td>75% (54/72)</td>
</tr>
<tr>
<td>Without SDRM</td>
<td>75% (43/58)</td>
</tr>
<tr>
<td>IIA or IIB virologic suppression</td>
<td>75% (38/51)</td>
</tr>
<tr>
<td>≥2 fully active agents in OBR</td>
<td>78% (32/41)</td>
</tr>
<tr>
<td>With dolutegravir</td>
<td>47% (26/54)</td>
</tr>
<tr>
<td>Without dolutegravir</td>
<td>89% (32/36)</td>
</tr>
</tbody>
</table>

**525 INITIAL SUPPLEMENTAL Dolutegravir DOSE in SECOND-LINE ART: A PHASE 2 RANDOMISED TRIAL**

Ying Zhao1, Rulan Griesel1, Zaayid Omar1, Bryony Simmons2, Andrew Hill3, Gert van Zyl1, Claire Keene4, Gary Maartens1, Graeme Meintjes1

1University of Cape Town, Cape Town, South Africa, 2University of Liverpool, Liverpool, United Kingdom, 3Stellenbosch University, Cape Town, South Africa, 4University of Oxford, Oxford, United Kingdom

**Background:** Dolutegravir concentrations are reduced by the induction effect of efavirenz, necessitating twice daily dolutegravir dosing when co-administered. Efavirenz induction persists for several weeks after stopping, which could select for resistance if switching occurred with unsuppressed HIV-1 RNA levels. We evaluated the need for a lead-in supplemental dolutegravir dose in adults failing first-line antiretroviral therapy (ART) with tenofovir-emtricitabine-efavirenz (TEE) switching to tenofovir- lamivudine-dolutegravir (TLD).

**Methods:** We conducted a randomised, double-blind, placebo-controlled, phase 2 trial in Khayelitsha, Cape Town, South Africa. Eligible patients had virologic failure (defined as two consecutive plasma HIV-1 RNA >1000 copies/ml) on first-line TEE. Participants were randomly assigned (1:1) to switch to TLD with a supplemental 50 mg dolutegravir dose or placebo taken 12 hours later for the first 14 days. The primary outcome was the proportion of participants with plasma HIV-1 RNA < 50 copies/ml at week 24. This study was not powered to formally compare arms.

**Results:** 130 participants were randomly assigned to receive TLD with supplemental dolutegravir (n = 65) or placebo (n = 65). Median age was 38 years (IQR 32 – 45), 69% female, and participants had a median of 85 months of experience with ART (IQR 50 – 119). The median baseline HIV-1 RNA was 4.0 log10 copies/ml (IQR 3.5 – 4.7) and 76% had baseline resistance to both tenofovir and lamivudine. Baseline characteristics were similar between arms. By week 24, one participant died and two were lost to follow-up. Proportions with virologic suppression at weeks 12 and 24 were similar between arms (Table 1). In the subgroup of participants with baseline resistance to both tenofovir and lamivudine, 76 (84%, 95% CI, 73 – 91%) of 90 participants had HIV-1 RNA < 50 copies/ml at week 24. There were 19 participants with HIV-1 RNA ≥50 copies/ml at week 24; 15/19 (79%) participants re-suppressed HIV-1 RNA < 50 copies/ml with enhanced adherence counselling. Grade 3 or 4 adverse events were similar in frequency between arms. None of the six participants (3 in each arm) meeting criteria for resistance testing developed dolutegravir resistance.

**Conclusion:** TLD in second-line ART was effective and well tolerated in both arms. Our findings do not support the need for initial dolutegravir dose adjustment in patients switching to TLD who failed first-line TEE.
Table 1: Summary of primary and certain secondary efficacy outcomes

<table>
<thead>
<tr>
<th>HIV-1 RNA &lt;40 copies/mL, n (%) [95% CI]</th>
<th>TLD + Daltegravir (n = 58)</th>
<th>TLD + Placebo (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention-to-treat (ITT) analysis*</td>
<td>55/64 (88%)</td>
<td>53/65 (84%)</td>
</tr>
<tr>
<td>Week 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intention to treat</td>
<td>55/64 (88%)</td>
<td>53/65 (84%)</td>
</tr>
<tr>
<td>Week 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intention to treat</td>
<td>55/64 (88%)</td>
<td>53/65 (84%)</td>
</tr>
</tbody>
</table>

* Primary endpoint analysis

526 DORAVIRINE: SAFETY, TOLERABILITY AND EFFICACY IN SOUTH AFRICAN WOMEN ON CONTRACEPTION

Nkosiphile Ndlovu,1 Ché K. Moshesh,1 Krishmaveni Reddy,1 Kimberly K. Scarsi,1 Thesla Palanee-Phillips,1 Nashon Yonge,1 Rena Patel,1 Shukri A. Hassan,1 Nazneem Cassim1, Nompumelelo Sigcu1

1Wits Reproductive Health and HIV Institute, Johannesburg, South Africa, 2University of Nebraska Medical Center, Omaha, NE, USA, 3University of Washington in Kenya, Nairobi, Kenya, 4University of Washington, Seattle, WA, USA

Background: Prior to local scale-up of new antiretroviral therapy (ART) for HIV treatment, exploring drug-drug interactions between ART and contraception is crucial. We are conducting EPIC (Evaluation of Pharmacokinetic drug-drug Interactions between Contraceptives and doravirine-containing ART), a parallel group pharmacokinetic study to examine interactions with doravirine (DOR)-containing ART (DOR+3TC+TDF) among women of reproductive potential living with HIV in Johannesburg, South Africa.

Methods: EPIC began in November 2021 and is ongoing. A ≥6-week lead-in period with oral DOR was required after azacitidine participants switched from their existing ART. Participants in the 3 intervention groups on DOR-containing ART concomitantly self-selected contraception: intramuscular depomedroxyprogesterone acetate (DMPA; Group 1), etonogestrel implant (ETG; Group 2), or copper intrauterine device (IUD; Group 3). ETG & IUD groups were compared to historical control groups using the same contraceptive method but on dolutegravir-containing ART, plus a fourth group to compare DMPA-users (dolutegravir + DMPA; Group 4). Participants were followed up for 12 (Groups 1 & 4) or 24 weeks (Groups 2 & 3). Early safety (measured by number & severity of adverse events (AE)), tolerability (measured by patient satisfaction & drug adherence), and efficacy (HIV viraemia as ≤40 copies/mL) are reported.

Results: Of 155 women screened, 87 have been enrolled: Group 1 (n=21), Group 2 (n=25), Group 3 (n=23) & Group 4 (n=22). The most common reasons for ineligibility were lack of HIV viraemia (n=43, 74%) and/or haemoglobin abnormality of grade 2 or higher (n=33, 52%) at screening. Of all AEs reported to date (n=162), 13 (8%) were attributable to DOR, of which headaches (n=4, 2%) or nausea (n=2, 1%) were most frequent. All AEs were reported at grade 1 except for one AE of diarrhoea in Group 2 at grade 2. Among the DOR groups, only 1 participant reported dissatisfaction with this ART, 99% were happy to continue it, and adherence by pill count was 96% for >76% adherence. All but 2 participants (7%) in the DOR groups maintained viral suppression at study exit. 1 participant (2%) or nausea (2%) in the DOR groups experienced adverse events (AEs) of grade 2 or higher (n=13; 22%) at screening. Of all AEs reported for ineligibility were lack of HIV viraemia (n=43, 74%) and/or haemoglobin abnormality of grade 2 or higher (n=13; 22%) at screening.

Discussion: Switching from first-line regimens to DOR-containing ART appears to be safe, tolerable, and efficacious in maintaining viral suppression in South African women concomitantly using hormonal contraceptives. These data support the viability of this option for those living in resource-limited settings.

Table 1: Safety, tolerability, and efficacy of doravirine-containing ART when concomitantly used with hormonal contraceptive methods, EPIC study, November 2022 to September 2023

<table>
<thead>
<tr>
<th>Group 1 (DOR+ETG)</th>
<th>Group 2 (DOR+3TC+TDF)</th>
<th>Group 3 (DOR+IUD)</th>
<th>Group 4 (DMPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of participants enrolled</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total number of adverse events reported</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Total number of participants with an adverse event reported</td>
<td>10</td>
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<td>Grading of adverse events</td>
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</tr>
<tr>
<td>Grade 1</td>
<td>30</td>
<td>30</td>
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</tr>
<tr>
<td>Grade 2</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Grade 3 or higher</td>
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<tr>
<td>Total adverse events attributable to DOR</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total adverse events attributable to DOR &amp; total at least Grade 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure mg Hg</td>
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<tr>
<td>N/A</td>
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<tr>
<td>ART satisfaction very satisfied</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Not satisfied</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Happy with contraceptive ART %</td>
<td>100</td>
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</tbody>
</table>

Conclusion: Given the low concentrations of DOR in urine, utility of a urine-based POCT would be limited to a readout of recent drug intake within 24 hours (i.e. 1 missed dose). By contrast, due to the persistence of TFV in urine, these data validate use of a urinary TFV threshold concentration of <1500 ng/mL (for TDF-based regimens) as a marker of 1-3 missed doses, for a POCT platform.
529 EARLY VIROLOGICAL OUTCOME OF DTG-BASED REGIMEN IN ADULT HIV-NAIVE PATIENTS INITIATED ON ART IN MALAWI: IMPACT OF tuổi NRTI RESISTANCE

Simeon Tuyishime,1 Bienvenu Niyongabo,2 Michel Gasana,1 Madeleine M. Uwungutse,1 Damascene J. Nirere,1 Benjamin Bizimana1, Gallician N. Rwibasira1, Samuel Malamba1,2

1Rwanda Biomedical Centre, Kigali, Rwanda, 2United States Agency for International Development, Kigali, Rwanda, 3Kigali University, Kigali, Rwanda, 4Kigali University, Kigali, Rwanda, 5University of Bern, Bern, Switzerland, 6Kwazulu-Natal Research Innovation and Sequencing Platform, Durban, South Africa

Background: In 2018, the Rwanda introduced DTG in the national ART rollout. In Malawi, DTG was introduced as part of the ART program. The aim of this study was to compare the early virological outcome of DTG-based regimens with other regimens in adult (≥15 years) ART-naive patients initiating ART in Malawi.

Methods: We conducted a retrospective analysis of virologic non-suppressed (≥1,000 copies/mL) patients who were switched to DTG-based ART regimen for functional DTG monotherapy and thus increase the risk of DTG resistance. As resistance testing at the time of the switch is generally unavailable, there are concerns that PLWH who switch to a DTG-based regimen with HIV replication could develop resistance to DTG. We report early viral load (VL) results from DTG SWITCH study in Malawi and Zambia, two ART programs with different switching policies.

Results and Conclusions: PLWH with unsuppressed VL tend to be higher in the DTG group compared to the non-DTG group (61.5% vs 58.7%, p<0.001). Unsuppressed VL was significantly lower in the DTG group (2.6% vs 4.7%, p<0.001) with an adjusted odd ratio (aOR) = 0.499 (95% CI 0.406 – 0.613) after adjusting for differences in age, sex, adherence, and province. Conclusion: PLWH initiated on DTG-based ART regimens have better virology outcomes than those initiated on DTG-based ART regimens. This is reassuring, since DTG has already been introduced in the national HIV treatment guideline as the first-line regimen option for newly diagnosed HIV+ clients.

EVALUATING THE IMPACT OF DTG-CONTAINING REGIMENS ON INFECTION CONTROL IN MALAWI AND ZAMBIA

Veronica Whitesell,1 Jaqueline Huwa,2 Geldert Chiwaya,2 Shameem Buleya,2 Joseph Chintedza1, Thokozani Kalua,2 Guy Muula,2 Esau Bandala1, Belinda Chiwata1, Carolyn Bolton Moore1, Stefanie Hossmann1, Roger D. Koyous1, Richard Lesells1, Gilles Wandeler1, Matthias Eggert1

1University of Bern, Bern, Switzerland, 2Lighthouse Trust, Lilongwe, Malawi, 3Center for Infectious Disease Research in Zambia, 4Center for Infectious Disease Research in Malawi (Lighthouse Trust, Lilongwe), 5University of Zurich, Zurich, Switzerland, 6Kwazulu-Natal Research Innovation and Sequencing Platform, Durban, South Africa

Background: WHO recommends Dolutegravir (DTG) as the third drug in first-line antiretroviral therapy (ART) for people living with HIV (PLWH). Some pre-existing NRTI resistance mutations could lead to “functional” DTG monotherapy and thus increase the risk of DTG resistance. As resistance testing at the time of the switch is generally unavailable, there are concerns that PLWH who switch to a DTG-based regimen with HIV replication could develop resistance to DTG. We report early viral load (VL) results from DTG SWITCH study in Malawi and Zambia, two ART programs with different switching policies.

Results and Conclusions: PLWH with unsuppressed VL tend to be higher in the DTG group compared to the non-DTG group (61.5% vs 58.7%, p<0.001). Unsuppressed VL was significantly lower in the DTG group (2.6% vs 4.7%, p<0.001) with an adjusted odd ratio (aOR) = 0.499 (95% CI 0.406 – 0.613) after adjusting for differences in age, sex, adherence, and province. Conclusion: PLWH initiated on DTG-based ART regimens have better virology outcomes than those initiated on DTG-based ART regimens. This is reassuring, since DTG has already been introduced in the national HIV treatment guideline as the first-line regimen option for newly diagnosed HIV+ clients.
**Conclusion:** The Malawi data indicate that PLWH switching with unsuppressed VL have a substantially higher risk of unsuppressed VL at 1 year than PLWH with suppressed VL at the time of the switch. The Zambian policy of only switching patients who were virologically suppressed at the last routine VL may reduce this risk.

**Table:** Virologic outcomes of PLWH at 1 year after routine switching to DTG-based first-line ART by viral load at switch, at two ART programmes in Malawi and Zambia.

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<thead>
<tr>
<th>Malawi</th>
<th>Zambia</th>
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<tr>
<td><strong>VL &lt; 400 copies/mL</strong></td>
<td><strong>VL &lt; 400 copies/mL</strong></td>
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<td><strong>VL missing at 1Y</strong></td>
<td><strong>VL missing at 1Y</strong></td>
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<td><strong>n (%)</strong></td>
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<td>Malawi</td>
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<tr>
<td><strong>VL &lt; 400 at BL</strong></td>
<td>1036</td>
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<tr>
<td>29 (2.7%)</td>
<td>24 (1.9%)</td>
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<tr>
<td>247 (18.8%)</td>
<td>87 (6.9%)</td>
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<tr>
<td>1512</td>
<td>1,288</td>
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<tr>
<td><strong>VL ≥ 400 at BL</strong></td>
<td>42</td>
</tr>
<tr>
<td>13 (22.5%)</td>
<td>11 (2.8%)</td>
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<tr>
<td>29 (29.3%)</td>
<td>37 (9.7%)</td>
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<tr>
<td>74</td>
<td>42</td>
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<tr>
<td><strong>RR (50%-95%)</strong></td>
<td>8.4 (2.7 to 15.7)</td>
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**Zambia**

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**531 TRENDS IN TDR TO FIRST-LINE ANTIMETRIVIRALS IN SPAIN: 2007-2021**

Laura Viñuela1, Adolfo de Salazar2, Ana Fuentes3, Adriana Pinto4, José Antonio iribarren5, Mar Masía1, Marta Montero3, Iker Falces-Romero6, Nuria Espinosa7, Begoña Baza8, Antonio Aguilera9, Joaquim Peraire10, Rebeca de Miguel11, Laura Viñuela11

*Presented at CROI by a nonauthor colleague*

**Background:** Treatment guidelines currently recommend resistance testing in the RT and protease of HIV as a part of the initial evaluation of HIV infected patients. Thus, it is of interest to evaluate the prevalence of drug resistance mutations (DRM) and, also of clinically relevant resistance. Here, we present data on trends in DRM and clinically relevant transmitted drug resistance to ARVs recommended for first-line treatment in Spain.

**Methods:** We analysed 7948 RT & Pro Fasta sequences from CoRIS (2007-2021). As Integrate resistance is not part of routine testing in naïve patients in Spain, we analysed 1975 integrase sequences. We evaluated the prevalence of RT/Pro and INSTI SDRMs using the CPR tools from Stanford. Clinically relevant resistance was evaluated using the Stanford Algorithm v9.1. We analysed periods for which significant changes in first line regimens recommended by the Spanish treatment guidelines (GESIDA) were provided.

**Results:** During the period 2007-2019, the prevalence of SDRMs were 3.5% NRTI, 4.3% NNRTI, 2.0% PIs, and 2.7% INI; in the last two years (2020-2021) we observed a similar trend for NNRTIs and NRTIs SDRM (3.8% NRTIs, 6.5% NNRTI), and a decrease in TDR to PIs (1%) and to INSTIs (0%). Clinically Relevant resistance to recommended GESIDA’s first line regimens showed no trend from 2007-2012 (always close to 1%), peaked in 2013-2014 due to the inclusion of Rilpivirine for 1st line in the Spanish recommendations, and lowered to below 3% for the years 2015-2019. For years 2020-2021, clinically relevant resistance to first line antiretrovirals in Spain was 0% for Dolutegravir, Bictegravir and Darunavir, 0.5% for 3TC/FTC; 1.2% for TDF/TAF, 1.7% for Abacavir, and 2.4% for Raltegravir.

**Conclusion:** While NNRTIs and NRTIs SDRM prevalence remained stable in Spain through 2007-2021, we observed a trend to a decrease in the levels of PIs and INIs SDRMs. Clinically relevant TDR to approved first line regimens is at low levels since 2016 to 2021. These findings support GESIDA’s recommendations on baseline resistance testing and test and treat strategies.

**532 WHAT INFLUENCES SWITCHING TO DTG/3TC VS B/F/TAF IN CLINICAL PRACTICE?**

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1Brigham and Women’s Hospital, Boston, MA, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3Touro Health, Lockport, NY, USA, 4Glided Sciences, Inc, Foster City, CA, USA, 5AlCHEMY Health Network, Los Angeles, CA, USA, 6Midway Immunology and Research Center, Fort Pierce, FL, USA, 7Philadelphia FIGHT, Philadelphia, PA, USA, 8Rush University Medical Center, Chicago, IL, USA, 9Care Resource, Miami, FL, USA

**Background:** Both B/F/TAF and DTG/3TC are recommended in treatment guidelines for both initial and switch therapy in people with HIV (PWH).

Understanding clinical and socio-demographic drivers of switching to DTG/3TC or B/F/TAF and how PWH vary by regimen is critical when comparing outcomes.

**Methods:** Retrospective study with TriHealth HIV Network EMR data.

Eligibility: ≥18 yrs, switched to B/F/TAF or DTG/3TC after DTG/3TC approval (4/2019-6/2022). Baseline (BL) characteristics were compared (X-square, t-test).

Logistic regression (LR) predicted probability of prescribing/dispensing DTG/3TC (propensity score; PS) given BL characteristics. Classification regression trees (CRT) and LR identified primary predictors of prescribing/dispensing DTG/3TC.

All shown comparisons were significant (p<.05).

**Results:** Of 6996 PWH, 1116 (16%) were prescribed DTG/3TC. PWH prescribed DTG/3TC differed in key characteristics: age ≥50 yrs (49 vs 38% B/F/TAF), commercial payer (57 vs 45%), VL viral suppression (VS < 200 copies/ml; 94 vs 92%), CD4 >200 cells/mm³ (84 vs 66%), eGFR < 60 mL/min/1.73m² (15 vs 6%), prior INSTI use (59 vs 36%), obese (BMI > 30 kg/m²; 32 vs 26%), hyperlipidemia (32 vs 28%), hypertension (34 vs 25%), osteoporosis (4 vs 2%), renal disease (10 vs 3%), alcohol (4 vs 6%), or substance use (5 vs 10%). Similar results were observed in the subset with dispensing data (n=3946; 13% DTG/3TC).

**Conclusion:** Baseline characteristics of PWH switching to DTG/3TC vs B/F/TAF were significantly different. B/F/TAF prescription was associated with factors that reflect HIV clinical parameters and adherence (e.g., VS, CD4< 200, substance use). By contrast, prescribing DTG/3TC was associated with renal toxicity and obesity. The results argue that although they are both guideline-recommended regimens, clinicians do not perceive them as equally appropriate for all patients. Accounting for channeling bias in observational studies evaluating outcomes is essential for interpreting differences between regimens.
ASSOCIATION OF WEIGHT GAIN ON DOLUTEGRAVIR WITH CHANGES IN ADHERENCE AND VIRAL LOAD

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AFRICOS Study Group
1Walter Reed Army Institute of Research, Bethesda, MD, USA; 2Walter Reed Army Institute of Research, Silver Spring, MD, USA; 3HIV Medical Research International, Kismay, Kenya; 4Walter Reed Army Institute of Research, Kinshasa, Congo; 5Walter Reed Army Institute of Research, Abuja, Nigeria; 6Walter Reed Army Institute of Research, Mbeya, Tanzania, 7Madero University Walter Reed Project, Kampala, Uganda

Background: Excess weight gain has been associated with dolutegravir-containing antiretroviral therapy (ART), especially among people living with HIV (PLWH) of female sex and Black African origin. If undesired, weight gain after initiating dolutegravir might impact adherence, with potential downstream effects on viral suppression. We sought to examine the relationship between changes in body weight (BW) and changes in adherence and viral load (VL) among PLWH switching to tenofovir disoproxil fumarate/lamivudine/dolutegravir (TLD).

Methods: The longitudinal African Cohort Study (AFRICOS) enrolls PLWH engaged in routine care at 12 PEPFAR-supported facilities in Kenya, Tanzania, and Uganda. We included participants who initiated TLD and had been on ART for ≥6 months prior and had visits within both 12 months before and 6-18 months after starting TLD, and were not pregnant. Pre/post-TLD initiation, we examined percent change in BW (≤1.5% [minimal change], >1.5% loss, >1.5% <5% gain, ≥5% gain [clinically significant]), change in self-reported antiretroviral adherence (0, 1-3 doses missed in past 30 days), and change in HIV-1 RNA VL category (≤50 c/mL [undetectable], 50-999 c/mL [suppressed], ≥1000 c/mL). The association of percent BW change with change in adherence and VL was examined using multivariable multinominal logistic regression models using no change in either outcome as the reference value.

Results: Among 1507 participants who started TLD from 10/2018-11/2021, median time from starting TLD to follow-up visit was 9 months (IQR: 7, 11). Pre/post-TLD, the prevalence of overweight/obesity increased from 33% to 38%, with 29% of all participants having a ≥5% BW gain. There were statistically significant (p=0.01) differences by sex in percent BW change, with largest differences for a ≥5% gain (females: 32%, males: 25%) and a >1.5% loss (females: 21%, males: 25%; 23% overall). Compared with a BW change ≤1.5%, BW changes ≥1.5%, including a ≥5% BW gain, were not significantly associated with changes in adherence or VL (figure). Results were similar when limiting to participants who were not overweight at baseline.

Conclusion: Clinically significant weight gain after switching to TLD may not be a major threat to short-term adherence or viral suppression among ART experienced PLWH in sub-Saharan Africa. Although this finding is reassuring, increasing cardiometabolic risk in the TLD era needs to be urgently addressed while simultaneously optimizing HIV treatment outcomes.

Associations* of changes in body weight with changes in adherence and viral load before and after starting TLD

PREDICTIVE VALIDITY OF A MEASURE OF BARRIERS TO ART ADHERENCE FOR HIV CARE (I-SCORE)

Kim Engler1, Serge Vicente1, David Lessard1, Karine Lacombe1, Dominic Chu1, Hayette Rougier2, Lucas Devlall2, Jean-Pierre Routy1, Alexandra De Pokomandy1, Marina Klein1, Bertrand Lebouche1

1Research Institute of McGill University Health Centre, Montreal, QC, Canada, McGill University, Montreal, QC, Canada, Saint-Antoine Hospital, Paris, France, Universite de Liege, Liege, France, McGill University Health Centre, Montreal, QC, Canada

Background: It is recommended to identify and address adherence barriers to antiretroviral therapy (ART) in routine HIV care. From a conceptual framework, we created a 7-item patient-reported measure (Interference-Score) to evaluate seven barrier domains (i.e. Thoughts/Feelings, Habits/Activities, Social situation, Economic status, Medication, Care, and Health). Both English and French versions were produced with stakeholder engagement and cognitive testing. Here, we assess the measure’s construct validity.

Methods: Recruited January to August 2022; people with HIV (PHIV) on ART in Canada and France completed the 7-item I-Score with 4 dependent variables at baseline (Time 1) and 4 weeks later (Time 2); dichotomized self-reported measures of adherence (past 7 days, past 30 days), intention to adhere, and HIV viral load. We conducted 3 types of analyses: 1) inter-item correlations (Spearman’s coefficients) to assess item redundancy at Time 1; 2) logistic regressions with all 7 items (covariates), with one model for each dependent variable, to assess each item’s significance; and 3) Receiver operating characteristic (ROC) curve analyses and appropriate reference values, to evaluate the 7-item models’ predictive capacity. Analyses 2) and 3) were performed using I-Score Time 1 items to predict the dependent variables at Time 1 and Time 2, respectively. Areas under the curve are reported with 95% bootstrap confidence intervals.

Results: Analyses included 265 PHIV at Time 1 and 154 at Time 2. Correlation coefficients ranged from 0.33 to 0.68; hence, no item was considered for elimination. Table 1 shows all other statistical analyses. The items (covariates) of “Thoughts”, “Habits”, “Health” and “Economic status” were significantly and independently associated with from 1 to 3 dependent variables. The ROC analyses showed the 7-item models’ predictive capacity to be “excellent” to “outstanding” for viral load, correctly classifying over 80% of respondents. The models were also “acceptable” for Adherence in the past 30 days and past 7 days, correctly classifying at least 72% of respondents.

Conclusion: While there was sample attrition from Time 1 to Time 2, no negative impact on the models’ predictive capacity was observed. The 7-item I-Score is a promising, simple, valid, and comprehensive tool to evaluate ART adherence barriers in care. Its items show limited redundancy, and the analyses provide strong evidence of concurrent and predictive validity for viral load.

Table 1. Results of the eight logistic regressions and ROC curve analyses for the 7-item I-Score.

US PATIENT PREFERENCES FOR LONG-ACTING HIV TREATMENT: A DISCRETE CHOICE EXPERIMENT

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Emory PREFER Team
1University of Washington, Seattle, WA, USA; 2Oregon Health and Sciences University, Portland, OR, USA, 3Centers for Disease Control and Prevention, Atlanta, GA, USA, 4Emory University, Atlanta, GA, USA

Background: Recent advances in long-acting antiretroviral therapy (LA-ART) could provide new options for HIV treatment and reduce adherence barriers, if regimens are acceptable to patients. We elicited preferences for key attributes of potential LA-ART regimens among people with HIV (PHIV) in the United States, focusing on four different treatment modalities (oral tablets, subcutaneous and
intranmuscular injections, and implants), product characteristics, and location of administration.

Methods: A discrete choice experiment was conducted among PWH aged ≥18 years recruited from HIV clinics in western Washington State and Atlanta, Georgia from 3/2021 to 6/2022. Participants responded to 17 choice scenarios, each with three options: two systematically generated hypothetical LA-ART regimens and a constant opt-out (their current daily oral treatment). LA-ART regimen descriptions included delivery mode, pain, dosing frequency, location, pre-treatment time undetectable, pre-treatment negative reaction testing, and late-dose leeway (i.e., flexibility in timing next dose). Implant frequency was constrained to be every 6 or 12 months, with mild or moderate pain. We used conditional logistic regression, with an interaction between delivery mode and pain, to estimate preference weights for all attribute levels.

Results: Seven hundred participants (350 at each site) enrolled, with median age 51 years (range 23-70); 70% identified as male, 24% as female, and 6% as non-binary/missing. Preference weights for each attribute level were summarized (Figure), with more preferred characteristics having higher preference weights. Across all participants, LA oral tablets were the only modality preferred over current daily oral treatment, with annual implants and injections the next most preferred. Longer time between doses was preferred, and administration at home was preferred to clinics, which were preferred to pharmacies. Attributes that impacted preferences less included oral lead-in treatment to achieve viral suppression or test for negative reactions and late-dose leeway around the prescribed dosing interval.

Conclusion: PWH in the United States may soon have several options for LA-ART. Our results suggest that LA oral tablets will be preferred by many patients over their current treatment, while implants and injections with longer duration may be acceptable to some patients. Future research should investigate sources of preference heterogeneity and actual uptake of and retention on products, when available. 

Figure: Long-acting antiretroviral treatment preference weights from conditional logistic regression.

HIV-1 RNA DECAY IN SEMEN AND RECTUM AFTER INITIATING THERAPY WITH DTG PLUS 3TC

Sofía C. Scevola1, Jordi Niubo2, Pere Domingo1, Guillermo Verdejo1, Adrià Currán4, Víces Diaz de brí0, Juan M. Tira bolschi, Sandra Morenilla1, Benito García1, Irene Soriano1, Daniel Podzamczer1, Arkaitz Imaz3

1Hospital Universitari Vall d’Hebron, Barcelona, Spain, 2Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, 3Hospitalet Universitari Vall d’Hebron, Barcelona, Spain, 4Hospital Universitario de Bellvitge, Llobregat, Barcelona, Spain, 5Institut Municipal de la Salut, Barcelona, Spain

Background: The study of HIV decay in genital fluids and rectum is important to assess the potential risk of sexual transmission after initiating ART. We observed similar HIV-1 RNA decay patterns in seminal plasma (SP) and rectal fluid (RF) with DTG+3TC and BIC/FTC/TAF (Iam et al. CROI 2022). The present study aims to evaluate differences in HIV-1 RNA decay in SP, RF, and blood plasma (BP) with the dual combination DTG+3TC.

Methods: This is a sub-study of a randomized pilot trial. Inclusion criteria were ART-naive male, BP HIV-1 RNA < 500,000 copies/mL, CD4 count >200 cells/μL, HBV negative and no resistance to the study drugs. Participants were randomized (2:1) to DTG+3TC 300 or BIC/FTC/TAF. HIV-1 RNA was measured in BP, SP and RF at baseline (BL), days 3, 7, 14 and 28, weeks 12 and 24 (LOQ: 20 c/mL or swab). This analysis is focused on the DTG+3TC group. HIV-1 RNA decline from BL at each timepoint was compared between compartments using the Friedman test. Linear mixed effects models were used to assess factors related with HIV-1 RNA decline.

Results: Of 406 participants between 2020-2022 (62% aged 50+, 60% male, 42% non-Hispanic Black), 47% reported at least one ART concealment behavior, 6% reported < 85% adherence, and 64% of the 308 participants who responded preferred LAI. ART concealment was associated with at-risk drinking (aOR 2.5 95% CI 1.4, 4.6), depression symptoms (aOR 2.2 95% CI 1.1, 4.2), incomplete disclosure to their close social network (aOR 3.1 95% CI 1.9, 5.0) or reporting not having a social network (aOR 3.3 95% CI 1.4, 7.8), and ≥85% ART adherence (aOR 0.3 95% CI 0.1, 0.9). ART concealment was not significantly associated with LAI preference in simple regression (OR 1.3 95% CI 0.8, 2.3).

Conclusion: Concealing ART to hide one’s HIV status was common and was associated with at-risk drinking, depression, incomplete disclosure to or lack of close social network, and reduced likelihood of optimal adherence. These findings show PWH may prefer receiving ART through mechanisms that ensure privacy. Future research can examine the impact of ART concealment on HIV outcomes like viral suppression and improve adherence among PWH concerned with disclosure of their HIV status.
538 ANTIVIRAL ACTIVITY OF LENACAPAVIR AGAINST HIV-2 ISOLATES
Robert A. Smith, Dani Raugi, Robbie Nixon, Moussa Seydi, Nicolas Margot, Christian Callebaut, Geoffrey Gottlieb

Background: Lenacapavir (LEN) is a first-in-class, multistage inhibitor of HIV-1 capsid function in clinical development that was recently approved in the European Union for use in adults with multidrug-resistant HIV-1 infection. LEN is highly potent against HIV-1 in vitro and maintains wild-type activity across HIV-1 isolates with resistance to all existing drug classes. In clinical trials, LEN has shown high levels of efficacy in people with HIV-1 who are treatment-naïve or treatment-experienced. However, a comprehensive characterization of the antiviral activity of LEN against HIV-2 is lacking. Herein, we studied the activity of LEN against a panel of HIV-2 isolates with or without resistance to existing drug classes.

Methods: The activity of LEN against HIV-1 and HIV-2 isolates from antiretroviral-naïve individuals was directly compared in two different assays: single-cycle infections of MAGIC-SA indicator cells and multicycle infections of an immortalized T cell line (CEM-NKR-CCR5-Luc). Drug-resistant HIV-2 variants with mutations in reverse transcriptase (RT) and integrase (IN) were tested for resistance to LEN in the single-cycle assay.

Results: In the single-cycle assay, LEN inhibited HIV-1 with a mean 50% inhibitory concentration (IC50) of 210 pM (range = 140–310 pM; n = 10 isolates). In comparison, the mean IC50 value for HIV-2 was 2.3 nM (range = 1.1–3.2 nM; n = 12 isolates), indicating an 11-fold decrease in the activity of LEN against HIV-2 compared with HIV-1. In the multicycle assay, a comparable difference in LEN activity between HIV-1 and HIV-2 was also noted, with mean IC50 values of 110 pM for HIV-1 (range = 67–196 pM; n = 4 isolates) and 1.8 nM for HIV-2 (range = 1.0–3.2 nM; n = 6 isolates). The presence of drug resistance mutations in HIV-2 RT and IN had no effect on LEN activity (fold-change in LEN IC50 = 0.73–1.2 relative to wild-type HIV-2RD09).

Conclusion: In our study, LEN was active against HIV-2 isolates with low-nanomolar activity, but was 11- to 16-fold less potent against HIV-2 in comparison to HIV-1, regardless of the presence of drug resistance mutations in HIV-2 RT or IN. As a result of this difference in potency, treating people with HIV-2 with a LEN-based regimen would require careful monitoring to assess virologic and immunologic responsiveness. These data provide information on the potential clinical utility of LEN in people with HIV-2 for whom treatment options are limited.

539 IMMUNO-VIROLOGICAL AND CLINICAL FOLLOW-UP OF HIV-2 PATIENTS RECEIVING BIC/FTC/TAF
Veronique A. Joly, Valentine M. Ferré, Mélanie M. Cresta, Charlotte Charpentier, Marc Digumber, Florence Damodé, Gilles Peytavin, Yazdan Yazdanpanah, Diane Descamps, Jade Ghozi

Background: HIV-2 infection remains a significant health problem in West Africa. Choices of antiretroviral drugs (ARVs) are limited but HIV-2 is fully susceptible to all nucleoside inhibitors (NRTIs) and integrase inhibitors (INSTIs). Bictegravir is active in vitro but no clinical data are available.

Methods: A retrospective study of patients (Pts) followed for HIV-2 infection in the Infectious Diseases Unit at Bichat Hospital, Paris, France and treated with BIC/FTC/TAF. Data were obtained from flow charts after patients’ written informed consent and were censored at September 1st 2022.

Results: Twenty-four Pts living with HIV-2 (PLHIV-2) received BIC/FTC/TAF, 14 women and 10 men. Median age was 56 yrs [IQR 50.2–60.2], median time since HIV-2 infection diagnosis was 19 yrs [IQR 8–23] and median nadir CD4 cell count 319/mm3 [IQR 174–432]. Zenith viral load was below 100 c/mL in 13 Pts and detectable in 11 Pts with a median value of 597 c/mL [IQR 513–567]. Five Pts were treatment-naïve and 19 were receiving ARVs with a median of 2 [IQR 1–3] previous regimens. ARVs preceding switch to BIC/FTC/TAF was a backbone of 2 NRTIs combined with darunavir in 5 Pts, raltegravir in 10 Pts, and dolutegravir in 4. Eight of these 19 pretreated patients had an history of treatment failure. At time of BIC/FTC/TAF initiation, median CD4 cell count was 580/mm3 [IQR 380–697]. Three Pts only, all naïve, had detectable viral load (57, 94 et 130 c/mL) with a viral load assay threshold of 40 c/mL. At time of evaluation, the median duration of BIC/FTC/TAF treatment was 21.7 months [IQR 12.9–30.4]. No Pt discontinued treatment. Viral load was < 40 c/mL in all Pts. Median CD4 cell count was 655/mm3 [IQR 495–800], p = 0.06 by Wilcoxon signed rank test when compared with CD4 count at time of BIC/FTC/TAF initiation.

Conclusion: In this observational study of PLHIV-2, BIC/FTC/TAF was well tolerated and efficient to achieve or maintain suppression of HIV-2 replication in all Pts. These results suggest that this BIC/FTC/TAF single-tablet-regimen is a valuable treatment option in PLHIV-2.

Furthermore, TAF has a reduced risk of renal toxicity compared to TDF and is a valuable treatment option in PLHIV-2.

540 ULTRA-LONG-ACTING BICTEGRAVIR NANOFORMULATIONS
Mohammad Ullah Nayyan, Srijaneet Das, Brady Sillman, Brandon Hanson, Siddappa Byarareddy, Howard E. Gendelman, Benson Edaga

Background: Antiretroviral therapy (ART) halts viral replication leading to reduced morbidity, mortality, and transmission of infection in people living with or at risk for HIV infection. However, therapeutic limitations remain. These include suboptimal adherence, adverse events, regimen tolerability, and viral resistance. To these ends, the need for ultra-long-acting (ULA) antiretroviral (ARV) formulations with extended duration of action cannot be overstated. Herein, we report the transformation of bictegravir (BIC) into prodrug formulations with apparent half-life extensions. The BIC prodrugs and corresponding nanocrystals were tested for their physicochemical and pharmacokinetic (PK) properties. The overarching goal was to create ULA BIC prodrug formulations with limited injection volumes and a shorter terminal phase PK tail.

Methods: Dimeric (DiBIC) and monomeric (MBIC, M2BIC, M3BIC) BIC prodrugs were synthesized by one-step dimerization and mono-esterification reactions. The synthesized prodrugs were nanoformulated by high-pressure homogenization. The formed aqueous solid drug nanocrystals were stabilized by non-ionic surfactants. Prodrug stability, particle size, homogeneity, and surface charge were assessed. Cellular uptake and retention, antiretroviral efficacy, and cytotoxicity were tested in primary human monocyte-derived macrophages (MDM). Following a single intramuscular (IM) injection, PK and biodistribution (BD) profiles were evaluated in Balb/c mice, SD rats, and rhesus macaques.
542 ZINC ADJUVANT TREATMENT IN SARS-CoV-2: A RANDOMIZED CLINICAL TRIAL

Silvia Gomez-Zorrilla, Juan Du, Elena Sendra, Merce Espona, Ana Sivero, Alicia Rodriguez-Alarcon, Claudia Navarro-Valls, Oriol Rins-Lozano, Esperanza Cahais-Ruano, Itziar Arrieta-Aldea, Alejandro Fierro-Villagas, Cristina Plata, Natalia Garcia-Giral, Ruben Vicente, Robert Guerra-Fernandez
Hospital del Mar, Barcelona, Spain

Background: The immune system is highly susceptible to changes in zinc levels and this might imply a different response against infection. Prior evidence suggests some benefit on viral infection prognosis after zinc supplementation. We aim to study the efficacy of zinc supplementation in SARS-CoV-2 infection outcomes.

Methods: This is an unicenter prospective, randomized clinical trial where unvaccinated individuals with moderate SARS-CoV-2 infection without end-organ failure were randomized to standard of care + oral zinc for 15 days (three times per day a tablet of 83 mg of Zn acetate equals to 75 mg of Zn element) in terms of age, gender and comorbidities nor in SoC were found between groups (Table 1). 14-day Mortality was 2.90% (2 participants) in the SoC group and none in zSoC (p = 0.005). No significant differences in changes in inflammatory markers were found among groups. No severe adverse events were observed during the study.

Conclusion: Daily zinc supplementation with 240 mg of zinc acetate for 14 days during the acute phase of SARS-CoV-2 infection resulted in lower rates of severity (less death and ICU admission) and faster clinical recovery along with shorter hospital stay.

Table 1. Population Characteristics at inclusion

<table>
<thead>
<tr>
<th></th>
<th>SoC</th>
<th>ZSoC</th>
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<tbody>
<tr>
<td>Age median (IQR)</td>
<td>52 (43-56)</td>
<td>53 (46-64)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>21 (65%)</td>
<td>20 (69%)</td>
</tr>
<tr>
<td>Active smoking, n (%)</td>
<td>6 (14%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>High Blood Pressure, n (%)</td>
<td>13 (35%)</td>
<td>9 (26%)</td>
</tr>
<tr>
<td>Type 2 Diabetes Mellitus, n (%)</td>
<td>5 (12%)</td>
<td>6 (17%)</td>
</tr>
<tr>
<td>COPD, n (%)</td>
<td>1 (2.7%)</td>
<td>1 (2.8%)</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
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Blood parameters

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<tr>
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<th>ZSoC</th>
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<tr>
<td>C- Reactive Protein mg/dL, median (IQR)</td>
<td>5.7 (2.3-9.9)</td>
<td>5.4 (2.3-8.4)</td>
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<tr>
<td>IL-6, median (IQR)</td>
<td>12.4 (2.2-63.5)</td>
<td>12.8 (4.3-63.7)</td>
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<td>D-Dimer (mg/L), median (IQR)</td>
<td>655 (241-630)</td>
<td>470 (250-500)</td>
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<td>Zinc mg/dL, median (IQR)</td>
<td>87 (81-114)</td>
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<td>PaO2/FiO2</td>
<td>275 (253-311)</td>
<td>257 (235-311)</td>
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<tr>
<td>NEWS-Score, mean (IQR)</td>
<td>9.85 (5)</td>
<td>9 (2.4)</td>
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Treatments

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<th>ZSoC</th>
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<tr>
<td>Dexamethasone, n (%)</td>
<td>87 (100%)</td>
<td>84 (100%)</td>
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<tr>
<td>Tocilizumab, n (%)</td>
<td>8 (20%)</td>
<td>7 (20%)</td>
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<tr>
<td>Low-Weight Heparin, n (%)</td>
<td>93 (89%)</td>
<td>91 (89%)</td>
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</table>

543 RETHINKING REMDESVIR: ENHANCING ANTI-SARS-CoV-2 ACTIVITY OF ORAL LIPID RVn PRODRUGS

Aaron Carlin1, James Beadle1, Alex Clark1, Kendra Gully1, Fernando Moreira1, Ralph Baric1, Rachel Graham1, Nadejda Vailaaeva1, Sandra Leibel1, William Bray1, Rachel McMillian1, Xing Quan Zhang1, Joyce Murphy1, Robert Schooley1, Karl Hostetter1
1University of California San Diego, San Diego, CA, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: SARS-CoV-2 evolution has contributed to successive waves of infections and severely compromised the efficacy of available SARS-CoV-2 monovalent antibodies. Decaying vaccine-induced immunity, vaccine hesitancy, and limited vaccine protection in older and immunocompromised populations further compromises vaccine efficacy at the population level. Early antiviral treatments, including intravenous remdesivir (RDV), reduce hospitalization and severe disease due to COVID-19. An orally bioavailable RDV analog could facilitate earlier widespread administration to non-hospitalized COVID-19 patients.

Methods: We synthesized monovalyl glycerol ether phospholipid analogs of GS-441524 (RVn), lysophospholipid analogs which allow for oral bioavailability and stability in plasma. We evaluated the in vivo efficacy of our lead compound, 1-octadecyl-2-0-benzyl-sn-glycerol-3-phospho-RVn (V2043), in an oral treatment model of murine SARS-CoV-2 infection. We then synthesized numerous phospholipid analogs of RVn and determined which modifications enhanced in vitro antiviral activity and selectivity. The most effective compounds against SARS-CoV-2 were then evaluated for antiviral activity against other RNA viruses.

Results: Oral treatment of SARS-CoV-2 infected BALB/c mice with V2043 (60 mg/kg once daily for 5 days, starting 12 hrs after infection) reduced lung viral load by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5. V2043 inhibited previous and contemporary SARS-CoV-2 Variants of concern (SARS-CoV-2) by more than 100-fold versus vehicle at day 2 and to below the LOD at day 5.
These compounds also showed enhanced antiviral activity against multiple contemporary and emerging RNA viruses. **Conclusion:** Collectively, our data support the development of RVn phospholipid prodrugs as oral antiviral agents for prevention and treatment of SARS-CoV-2 infections and as preparation for future outbreaks of pandemic RNA viruses.

**544 SARS-CoV-2 ANTIVIRAL ACTIVITY OF ZOTATIFIN, A HOST-TARGETING eIF4A INHIBITOR**

Sam Sperry, Peggy Thompson, Craig Stumpf, Gary Chiang, Stephen Worland, Amy Patrick
eFFECTOR Therapeutics, Inc., Solana Beach, CA, USA

**Background:** Zotatifin (eFT226) is a potent and selective inhibitor of eukaryotic initiation factor 4A (eIF4A), a host RNA helicase required for SARS-CoV-2 replication. Zotatifin selectively inhibits translation of ribonucleic acids (RNAs) containing specific short polypurine motifs in their 5-prime (5’)-regions. Two such highly conserved motifs are found in the SARS-CoV-2 genome. Zotatifin is currently being evaluated in a Phase 1b dose escalation study in 36 patients with mild to moderate COVID disease. In this in vitro study, we evaluated the selectivity of zotatifin’s inhibition of SARS-CoV-2 translation, the antiviral activity of zotatifin alone against different human coronaviruses and the antiviral activity of zotatifin in combination with other antivirals against SARS-CoV-2.

**Methods:** The selectivity of zotatifin for viral translation was evaluated in a cell-based reporter assay wherein luciferase translation was driven by 5’-sequences from SARS-CoV-2 or tubulin, a housekeeping gene. The antiviral activity of zotatifin was evaluated against SARS-CoV-1, SARS-CoV-2 variants (Wash/1/2020 (ancestral), delta, omicron BA.2), MERS-CoV and HCoV-299E in primary or established cell lines using cytopathic effect or infectious virus as endpoints. The antiviral activity of zotatifin in combination with remdesivir, N-hydroxyctydine (NHC), active nucleoside analogue metabolite of molnupiravir, nirmatrelvir, baricitinib or sotrovimab was evaluated against SARS-CoV-2 and analyzed by the method of Pritchard and Shipman.

**Results:** Zotatifin inhibited the translation of the SARS-CoV-2 luciferase reporter construct with a mean IC50 of 3 nM and was ~14-fold less potent in inhibiting the tubulin reporter construct. Zotatifin potently inhibited the replication of all human coronaviruses tested with 50% effective concentrations (EC50) ranging from 0.016 to 37.3 nM. The 50% cytotoxic concentration (CC50) supports further evaluation in human clinical trials. Harmine induced loss of HIV-1 structural proteins. These compounds also showed enhanced antiviral activity against all human coronaviruses tested. Zotatifin has physicochemical and pharmacokinetic (PK) properties suitable for convenient, single subcutaneous (sc) injection. This study assessed the safety, antiviral activity, and PK of zotatifin in non-hospitalized patients (pts) with mild/moderate COVID.

**Methods:** PROPEL is a randomized, placebo-controlled, double-blind study in non-hospitalized pts with mild/moderate COVID. At randomization, pts must have had a SARS-CoV-2 positive test within 7 days and at least 1 COVID symptom. Pts were randomized (2:1) to zotatifin or placebo sc in 3 cohorts of 12 pts each. Cohort 1, 2 and 3 received a single dose (SD) of zotatifin of 0.01, 0.02 and 0.035 mg/kg or matching placebo. Safety (adverse event (AE) and laboratory tests), antiviral activity (mid-turbinate nasal swabs and saliva), and plasma PK were collected over 30 days. The primary endpoint was safety; key secondary endpoints included SARS-CoV-2 viral load (VL) and PK. The study was not powered for statistical inferential testing.

**Results:** 36 pts were enrolled across all three cohorts and completed a 30-day follow up. Data is currently available for pts in cohorts 1 and 2, 18 and 6 of whom received zotatifin and placebo, respectively. Baseline characteristics were comparable between groups. The most common AE was erythema at injection site in cohort 1 (44%) and cohort 2 (89%), vs. 0% in the zotatifin and pooled placebo groups, respectively. Other AE frequencies were comparable between zotatifin and placebo and no serious AEs were reported. The concentration-time profile of zotatifin from cohorts 1 and 2 following sc administration was similar to that reported previously following IV administration, demonstrated a terminal elimination half-life (t1/2) of ~4 days, high steady-state volume of distribution (Vss) of 31 L/kg, and low plasma clearance (Cl) of 3.9 mL/min/kg. A faster time to viral RNA undetectability was observed with zotatifin vs. placebo (see Fig 1. Not statistically significant).

**Conclusion:** Zotatifin was safe, well tolerated and demonstrated a trend in clinical antiviral activity in patients with mild to moderate COVID which supports further clinical development. Zotatifin sc route of administration supports a point of care treatment for COVID.

### Time to Viral Load Undetectability (VLU)

#### Nasal Swab

**546 WITHDRAWN**

**545 A PHASE 1B STUDY OF ZOTATIFIN FOR THE TREATMENT OF MILD TO MODERATE COVID (PROpel)**

Harry Malech1, Almena Free1, Enola Okonkwo1, Patricia Littel1, Nicole Rooths2, Priscilla Quackenbush1, Martha Marquesen1, Michael Hodges2, Amy Patrick1, Sam Sperry1, Bin Yao1, Nawaid Rana1, Doug Warner1

1National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 2Pinnacle Research Group, LLC, Anniston, AL, USA, 3Tampa General Hospital, Tampa, FL, USA, eFFECTOR Therapeutics, Inc., Solana Beach, CA, USA

**Background:** Zotatifin (eFT226) is a potent and selective inhibitor of eukaryotic initiation factor 4A (eIF4A), a host RNA helicase required for SARS-CoV-2 replication. In vitro, zotatifin demonstrates broad spectrum antiviral activity

### Delta

![Graph](Image)

<table>
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<tr>
<th>Drug</th>
<th>Log10(Drug)</th>
<th>zotatifin</th>
<th>remdesivir</th>
<th>molnupiravir</th>
<th>NHC</th>
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### Omicron BA.2

![Graph](Image)

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**Results:** The selectivity of zotatifin for viral translation was evaluated in a cell-based reporter assay wherein luciferase translation was driven by 5’-sequences from SARS-CoV-2 or tubulin, a housekeeping gene. The antiviral activity of zotatifin was evaluated against SARS-CoV-1, SARS-CoV-2 variants (Wash/1/2020 (ancestral), delta, omicron BA.2), MERS-CoV and HCoV-299E in primary or established cell lines using cytopathic effect or infectious virus as endpoints. The antiviral activity of zotatifin in combination with remdesivir, N-hydroxyctydine (NHC), active nucleoside analogue metabolite of molnupiravir, nirmatrelvir, baricitinib or sotrovimab was evaluated against SARS-CoV-2 and analyzed by the method of Pritchard and Shipman.

**Results:** Zotatifin inhibited the translation of the SARS-CoV-2 luciferase reporter construct with a mean IC50 of 3 nM and was ~14-fold less potent in inhibiting the tubulin reporter construct. Zotatifin potently inhibited the replication of all human coronaviruses tested with 50% effective concentrations (EC50) ranging from 0.016 to 37.3 nM. The 50% cytotoxic concentration (CC50) value for zotatifin was 250 to >100,000 nM, yielding selectivity indices of 7 to >6250. Zotatifin was ~20 to >100-fold more potent than remdesivir, nirmatrelvir or NHC (figure) and demonstrated additive interactions when combined with remdesivir, NHC, nirmatrelvir, baricitinib or sotrovimab in vitro.

**Conclusion:** The potent broad-spectrum activity of zotatifin against a variety of human coronaviruses and additive activity when combined with different anti-SARS-CoV-2 antivirals highlight the advantages of eIF4A as a target and warrant further evaluation in human clinical trials.

Gytopathic effect (CPE) inhibition assay in Vero E6 cells infected with SARS-CoV-2 variants (delta or omicron BA.2)

#### Delta

![Graph](Image)

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<th>remdesivir</th>
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#### Omicron BA.2

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547 SMALL MOLECULE INHIBITOR FOR BLOCKING SARS-CoV-2 ENTRY
Enming Xing, Yexiu Zhang, Rajni Kant Shukla, Jianrong Li, Valarmathy Murugiaahl, Xiaolin Cheng, Pui Kai Li, Amit Shama
Ohio State University, Columbus, OH, USA

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel and highly pathogenic coronavirus and is the causative agent of COVID-19, an ongoing pandemic that has posed a serious threat to public health and global economy. Thus, there is a pressing need for therapeutic interventions that target essential viral proteins and regulate virus spread and replication. To invade the host cell, the receptor-binding domain (RBD) of SARS-CoV-2 Spike protein binds to the host cell’s ACE2 receptor, followed by cleavage events that allow the Spike protein to fuse with the host cell membrane. Thus, the essential role of Spike protein in ACE2 receptor binding and viral fusion makes it a prime target for therapeutic interventions.

Methods: We performed molecular docking and molecular dynamics (MD) simulation-based virtual screening against SARS-CoV-2 RBD/ACE2 interface using a commercial library of 93,835 drug-like compounds. Compounds with promising docking poses and scores were selected for further MD simulation refinement, from which ten lead compounds were identified. Antiviral potencies of ten lead compounds were evaluated against lentiviral vectors pseudotyped with SARS-CoV-2 Spike to down select to a single lead compound, “SA4”. ELISA-based assays were employed to determine the binding affinities of SA4 to recombinant SARS-CoV-2 RBD. Antiviral potential of SA4 was validated against genuine SARS-CoV-2 in a BSL3 setting.

Results: We identified SA4 as a candidate small molecule, which inhibited SARS-CoV-2 pseudovirus entry with IC50 value of ~18 µM. We determined that SA4 binds RBD with a Kd of ~20 µM. Using cells engineered to express ACE2 and cells that express physiological levels of ACE2, we found that SA4 inhibited SARS-CoV-2 pseudovirus entry at both engineered and physiological ACE2 levels. We validated the antiviral potential of SA4 against genuine SARS-CoV-2 and HGCO-NL63. Lastly, we demonstrated antiviral potential of SA4 against four SARS-CoV-2 variants of concern (α, β, γ, and δ).

Conclusion: Using virtual screening, we identified SA4 as the promising hit compound which displayed inhibitory activities against SARS-CoV-2 entry and its four variants of concern. Thus, our study will pave the way for further development of small molecules for therapeutic targeting of SARS-CoV-2 to combat the COVID-19 pandemic.

548 PLITIDEPSIN IS A BROAD-SPECTRUM ANTIVIRAL SHAPING THE CELL PROTEOSTATIC BALANCE
Daniel Perez-Zsolt1, Elisha Molina Molina1, Joan Josep Bech-Serra2, Roger Badia1, Eva Rivero-Muñoz1, Sachse Martin3, Sandra Franco2, Pablo Avilés1, Miguel A. Martínez2, Roger Paredes1, Bonaventura Clotet4, Cristina Risce1, Ester Ballana1, Carolina De la Torre1, Nuria Izquierdo-Useros1
Broad-Spectrum Antivirals Study Group
1InCaixa AIDS Research Institute, Barcelona, Spain, 2Josep Carreras Leukemia Research Institute, Barcelona, Spain, 3Centro Nacional de Biotecnología, Madrid, Spain, 4PharMaco, Madrid, Spain

Background: Different viruses employ similar pathways for replication, revealing key intracellular hotspots to target with host-directed therapies and achieve a broad-spectrum antiviral activity. Plitidepsin is a clinically approved antitumoral agent that blocks the elongation factor eEF1A required for protein translation. This drug counteracts SARS-CoV-2 replication and shows a favorable safety profile in COVID-19 patients. Yet, the precise antiviral mechanism of action of plitidepsin remains unknown.

Methods: Here we used a deep quantitative proteomic analysis to measure the impact of plitidepsin on the proteome of SARS-CoV-2-infected Vero E6 cells. This was complemented with transmission electron microscopy assays, which unraveled the subcellular and morphological changes associated to plitidepsin treatment. In addition, we performed functional in vitro assays to dissect the antiviral activity of plitidepsin against SARS-CoV-2 and other viruses.

Results: We found that this drug inhibited the synthesis of all SARS-CoV-2 proteins in a dose-dependent manner. These included the R14B polypeptides, which facilitate the synthesis of non-structural proteins involved in the formation of double membrane vesicles (DMV) required for viral replication. Plitidepsin reduced DMV formation and the morphogenesis of new viruses, having a greater impact on viral than on host proteins. Less than 14% of the cellular proteome was significantly affected by plitidepsin, inducing the up-regulation of key molecules associated with protein biosynthesis, such as the translation initiation factors eIF4A2 and eIF2S3. Therefore, plitidepsin arrested virus replication even after the onset of viral protein production. At 24 h post-treatment, supernatant (TCID50). Transient co-expression of four SARS-CoV-2 structural proteins (N, M, S, E) to produce virus-like particles (VLPs) was used to study the effect of PAV-104 on viral assembly. Drug resin affinity chromatography was used to target a subset of host protein assembly machinery in a manner specific to viral assembly with minimal host toxicity. The chymotrypsin showed broad activity against respiratory viral pathogens, including Orthomyxoviridae, Paramyxoviridae, Adenoviridae, Herpesviridae, and Picornaviridae, with low susceptibility to evolutionary escape. Here, we investigated the capacity of PAV-104 to inhibit SARS-CoV-2 replication in human airway epithelial cells (AECs).

Methods: Dose-dependent cytotoxicity of PAV-104 in Calu-3 cells was determined by MTT assay. Calu-3 cells were infected with SARS-CoV-2 isolate USA-WAI1/2020 (MOI=0.01). Primary AECs were isolated from healthy donor lung transplant tissue, cultured at air liquid interface (ALI), and infected with SARS-CoV-2- Gamma, Delta, and Omicron variants (MOI=0.1). SARS-CoV-2 replication was assessed by RT-PCR quantitation of the N gene, immunofluorescence assay (IFA) of nucleocapsid (N) protein, and titration of supernatant (TCID50). Transient co-expression of four SARS-CoV-2 structural proteins (N, M, S, E) to produce virus-like particles (VLPs) was used to study the effect of PAV-104 on viral assembly. Drug resin affinity chromatography was performed to study the interaction between PAV-104 and N. Glycerol gradient sedimentation was used to assess N oligomerization. Total RNA-seq and the REACTOME database were used to evaluate PAV-104 effects on the host transcriptome.

Results: PAV-104 reached 50% cytotoxicity in Calu-3 cells at 3732 nM (Fig.1A). 50 nM PAV-104 inhibited >99% of SARS-CoV-2 infection in Calu-3 cells (p < 0.01) and in primary AECs (p < 0.01) (Fig.1B). PAV-104 specifically inhibited SARS-CoV-2 post entry, and suppressed production of SARS-CoV-2 VLPs without affecting viral protein synthesis. PAV-104 interacted with SARS-CoV-2 N and...
interfered with N oligomerization. Transcriptome analysis revealed that PAV-104 treatment reversed SARS-CoV-2 induction of the interferon and “maturation of nucleoprotein” signaling pathways.

**Conclusion:** PAV-104 is a pan-respiratory virus small molecule inhibitor with promising activity against SARS-CoV-2 in human airway epithelial cells that should be explored in animal models and clinical studies.

Figure 1. PAV-104 decreases SARS-CoV-2 replication in airway epithelial cells.

**SAFETY AND PHARMACOKINETICS OF A NOVEL DOUBLE PRODRUG ASC10 IN HEALTHY SUBJECTS**

Yunqing Qiu, Jian Liu, You Zhai, Qingwei Zhao, Yuemei Yan, Handan He, Jinzi J. Wu

**Background:** ASC10 is an oral double prodrug of the active antiviral ribonucleoside analog, ASC10-A (also known as β-D-N4-hydroxycytidine), which is a potent inhibitor of SARS-CoV-2. ASC10 is rapidly metabolized into ASC10-A in vivo after oral dosing. Here, we report the results of the first-in-human, phase 1 study to determine the safety, tolerability, and pharmacokinetics (PK) of ASC10 in healthy subjects, and to assess the food effect on the pharmacokinetics.

**Methods:** This study included 2 parts. Part 1 (multiple-ascending-dose) consisted of 6 cohorts (8 or 12 subjects per cohort). Eligible subjects were randomized in a 3:1 ratio to receive either twice-daily (BID) doses of 50 to 800 mg ASC10 in the fed state followed by 800 mg in the fasted state, or vice versa, with a 7-day washout period between doses. PK blood samples were collected and measured for ASC10-A along with ASC10 and molnupiravir. Safety assessments including monitoring of adverse events (AEs), measurement of vital signs, clinical laboratory tests, and physical examinations.

**Results:** ASC10-A was the major circulating metabolite (>99.94%) in subjects after oral dosing of ASC10. ASC10-A appeared rapidly in plasma, with a median T\textsubscript{max} of 1.00 to 2.00 h, and declined with a geometric t\textsubscript{1/2} of approximately 1.10 to 3.04 h. After multiple dosing for 5.5 days, both C\textsubscript{max} and AUC of ASC10-A increased in a dose-proportional manner from doses of 50 to 800 mg BID without accumulation. ASC10-A in the fed state occurred slightly later, with a median of 3.99 h postdose versus 2.00 h (fasted state). However, C\textsubscript{max} and AUC were very similar or the same between fed and fasted states. Thus, administration of ASC10 with food is unlikely to have an effect on exposure.

**Conclusion:** Results of this study showed that ASC10 was well tolerated, and the increase in plasma exposure of ASC10-A was dose proportional across the range of doses tested with no accumulation and no food effect. 800 mg ASC10 BID is selected for further studies in patients infected with SARS-CoV-2.

**MECHANISM AND RESISTANCE STUDIES OF SARS-CoV-2 MPRO INHIBITOR POMOTRELVIR (PBI-0451)**

Xiao Tong, Laura Stevens, Walter Keung, Lee Arnold, Urs Lopatin, Mark Denison, Ann Kwong

**Background:** The unprecedented scale of the COVID-19 pandemic and rapid evolution of SARS-CoV-2 variants underscores the need for broadly active inhibitors with a high barrier to resistance. The coronavirus main protease (Mpro) is an essential viral enzyme required for viral polyprotein processing and is highly conserved across human coronaviruses. Pomotrelvir (PBI-0451) is a novel Mpro inhibitor currently completing phase 2 clinical trial. Here we describe the mechanism of action, broad activity against SARS-CoV-2 clinical isolates, combination studies with other SARS-CoV-2 inhibitors and favorable resistance profile of pomotrelvir.

**Methods:** The kinetic parameters of pomotrelvir M\textsuperscript{MM} inhibition and its interaction with nirmatrelvir were determined in a kinetic protease assay. The IC\textsubscript{50} of pomotrelvir on mutant Mpro proteins were measured in an endpoint M\textsuperscript{MM} assay. Combination studies of pomotrelvir with remdesivir and molnupiravir were carried out in A549-hACE2 cells infected with SARS-CoV-2 NLuc virus. Activity against SARS-CoV-2 clinical variants was assessed by infection of A549-ACE2-TMPRSS2 cells followed by immunostaining of the viral nucleocapsid protein.

**Results:** Pomotrelvir is a potent competitive inhibitor of SARS-CoV-2 M\textsuperscript{MM} (K\textsubscript{i} = 2.7 nM). Binding of pomotrelvir and the M\textsuperscript{MM} inhibitor nirmatrelvir to the active site is mutually exclusive. In the SARS-CoV-2 NLuc assay, pomotrelvir is additive when combined with remdesivir or molnupiravir, two nucleoside analogs targeting viral RNA synthesis. When the effect of M\textsuperscript{MM} substitutions previously selected in a resistance study of pomotrelvir were analyzed in an enzyme assay, only Mpro_N133H showed a significant increase in IC\textsubscript{50} (45-fold). The catalytic efficiency of M\textsuperscript{MM} N133H is reduced by 10-fold and the recombinant virus SARS-CoV-2 (WA1) N133H is not viable, suggesting that N133H has lower replicative fitness. Lastly, pomotrelvir exhibits broad activity against all SARS-CoV-2 clinical isolates tested to date, including five omicron variants.

**Conclusions:** PBI-0451 is a potent competitive inhibitor of SARS-CoV-2 M\textsuperscript{MM} and is broadly active against SARS-CoV-2 clinical isolates including omicron variants. Results from inhibitor interaction studies support the potential combination of pomotrelvir with remdesivir and molnupiravir but not nirmatrelvir. Enzymatic characterization of in vitro selected pomotrelvir resistant variants indicates they either confer no resistance or have reduced fitness.

**SARS-CoV-2 E-PROTEIN VIROPORIN INHIBITOR BIT225 ACTIVE IN hACE2 TRANSGENIC MICE**

Michelle Miller, Gary Ewart, Michael Bobardt, Bo Benzen, Audrey Thomson, Klaus Klumpp, Stephen Becker, Mette Rosenkilde, Gallay Philippe

**Background:** The CoV-2 envelope (E) protein plays an important role in virus assembly, budding, immunopathogenesis and disease severity. E protein has ion channel activity, is located in Golgi and ER membranes of infected cells and is associated with inflammasome activation and immune dysregulation. Here we report that BIT225, an investigational HIV clinical compound, inhibits E protein channel activity and Xenopus TMEM16A.

**Results:** BIT225 inhibited ion channel activity of E-protein, but not that of TMEM16A in Xenopus oocytes. K18-hACE2 transgenic mice were infected intranasally with 10\textsuperscript{5} pfu SARS CoV-2 (US-WA1/2020) and dosed orally twice daily with BIT225 for up to 12 Days. Dosing was initiated 12 h pre-infection or 24 h or 48 h after infection.

**Methods:** SARS-CoV-2 E protein ion channel activity and XenoPus TMEM16A were measured in Xenopus oocytes. K18-hACE2 transgenic mice were infected intranasally with 10\textsuperscript{5} pfu SARS CoV-2 (US-WA1/2020) and dosed orally twice daily with BIT225 for up to 12 Days. Dosing was initiated 12 h pre-infection or 24 h or 48 h post-infection. Disease parameters measured were survival, body weight, viral RNA by qPCR and infectious virus titre (plaque assay) in lung tissue homogenates and serum. In addition, levels of pro-inflammatory cytokines (IL-6, IL-1α, IL-1β, TNFa & TGFβ, MCP-1) were measured in lung and serum samples.

**Results:** BIT225 inhibited ion channel activity of E-protein, but not that of TMEM16A in XenoPus oocytes. BIT225 dosed at 300mg/kg BID for 12 days starting 12 h pre-infection completely prevented body weight loss and
mortality in SARS-CoV-2 infected K18 mice (n=12), while all vehicle-dosed animals reached a mortality endpoint by day 9 across two studies (n=12). Figure 1 shows results from a time of addition study: When treatment with BIT225 started at 24 h post-infection, body weight loss and mortality was also prevented (100% survival, n=5). In the group of mice where treatment started at 48 h after infection, body weight loss and mortality were prevented in 4 of 5 mice. Treatment efficacy was associated with significant reduction in lung viral load (3.5 log$_{10}$) virus titer (4000 pfu/ml) and lung and serum cytokine levels. Conclusion: BIT225 is an inhibitor of SARS-CoV-2 E-protein viroporin activity. In the K18 model BIT225 protected mice from weight loss and death, inhibited virus replication and reduced inflammation. These effects were noted when treatment with BIT225 was initiated before or 24-48 hours after infection and validate viroporin E as a viable antiviral target and support the clinical study of treatment with BIT225 in treatment of SARS-CoV-2.

FIGURE 1: BIT225 Time of Addition Study: Time courses for (A) body weight and (B) mortality in SARS-2 infected K18-hACE2 mice (n=5 per group).

553 NAFAMOSTAT AS NASAL CHEMPROPHYLAXIS FOR SARS-CoV-2 IN HAMSTERS

Megan Neary1, Joanne Sharp1, Eduardo Gallardo-Toledo1, Joanne Herriott1, Edyta Kijak1, Chloe Bramwell1, Helen Cox1, Lee Tatham1, Paul Curley1, James Hobson1, Steve Rannard1, Anja Kipar2, James Stewart1, Andrew Owen1

1University of Liverpool, Liverpool, United Kingdom, 2University of Zurich, Zurich, Switzerland

Background: Chemoprophylaxis is a critical tool for many infectious diseases, and in COVID-19 may have particular benefit for vulnerable patients that do not maximally benefit from vaccination. Nafamostat inhibits TMPRSS2, which catalyses a critical cell entry pathway for SARS-CoV-2. This study sought to assess efficacy of intranasal nafamostat against airborne transmission of SARS-CoV-2 in Syrian Golden hamsters.

Methods: Male hamsters were intranasally administered water or 5 mg/kg nafamostat in water twice daily for 5 days (sentinels). One day after treatment initiation, sentinels were co-housed with an untreated hamster that was intranasally inoculated with 1 × 10^6 pfu of Wuhan SARS-CoV-2 (donor). Sentinels were separated from the donor by a perforated divider, allowing airflow between zones but not contact. Hamsters were weighed and throat-swabbed to assess nasal transmission. No severe adverse events were reported. Thus, the nasal spray was validated viroporin E as a viable antiviral target and support the clinical study of treatment with BIT225 in treatment of SARS-CoV-2.

Results: SARS-CoV-2 viral RNA was significantly lower in the nasal turbinates of nafamostat-treated hamsters compared to water-treated controls (P = 0.012; Figure 1). Within the lung, SARS-CoV-2 RNA was undetectable in the nafamostat-treated hamsters, but was detectable in the water-treated controls. Viral RNA was undetectable in the swabs of the nafamostat-treated hamsters at all timepoints, but was quantifiable in the water-treated control group from day 3. Body weight of the nafamostat-treated hamsters was significantly lower (P < 0.001) than in the water-treated animals throughout. SARS-CoV-2 viral RNA was detectable in the donor hamsters lung, nasal turbinates and swab samples confirming validity of the experiment.

Conclusion: This study demonstrated a protective effect of intranasal nafamostat against airborne SARS-CoV-2 transmission in Syrian golden hamsters. A phase Ia study of intravenously administered nafamostat yielded no evidence of clinical efficacy in hospitalised patients, but further investigation of intranasally administered nafamostat in a prophylactic setting may be warranted.

Figure 1: SARS-CoV-2 viral RNA concentration quantified from the nasal turbinates of the donor, nafamostat or water treated hamsters.

554 PHASE 1 CLINICAL TRIALS OF A Q-GRIFFITHSIN NASAL SPRAY FOR SARS-CoV-2 PROPHYLAXIS

Henry W. Nabella1, Maryam Zahir1, Amanda Lasnik1, Elizabeth D. Cash1, Xiaoyong Wu1, Shesh Rai1, Kathleen Kitterman1, Lin Wang1, Sranan Patel1, Lisa Rohan1, Sharon L. Hillier1, Nobuyuki Matoba1, Kevin L. Potts1, Gerald Dryden1, Kenneth E. Palmer1

1University of Louisville, Louisville, KY, USA, 2University of Cincinnati, Cincinnati, OH, USA, 3University of Pittsburgh, Pittsburgh, PA, USA

Background: The currently approved vaccines do not induce sterilizing immunity against SARS-CoV-2 infection, and immunity wanes over time. A robust broad spectrum topical prophylaxis strategy could protect vulnerable populations in the face of continuous evolution of SARS-CoV-2. The algal antiviral lectin Griffithsin (GRFT), and an engineered oxidation-resistant variant Q-GRFT have robust entry inhibitory activity against SARS-CoV-variants of concern, in addition to other respiratory viruses with pandemic potential. We designed a nasal spray to deliver Q-GRFT to the upper respiratory tract mucosa for on-demand use as a broad-spectrum prophylactic. Two clinical trials (Phase 1a and 1b) were conducted to assess safety, tolerability, and pharmacokinetics of Q-GRFT nasal spray in healthy adults.

Methods: Healthy adult volunteers were enrolled in a Phase 1a double blinded, randomized study to receive a single dose of either intranasal Q-GRFT (3.0 mg, 2 sprays per nostril) or placebo at 2:1 ratio. Following a safety review, the Phase 1b study was initiated. Eleven volunteers in Group 1 received 3.0 mg once daily, for 7 days. After a safety review, 11 volunteers in Group 2 received a total of 6.0 mg Q-GRFT (3.0 mg twice daily for 7 days). Topical Q-GRFT concentrations were measured by ELISA in collected nasal and nasopharyngeal fluids. Drug levels in plasma were assayed to determine systemic exposure. Viral neutralization cytotoxic effect (CPE) assays were performed against SARS-CoV-2 Omicron BA.5 and MERS-CoV.

Results: Eighteen adults (24-54 years; Males 58.3%, Females 41.7%; 12 Q-GRFT, 6 Placebo), and 22 adults (aged 23-59 years; Males 52.4%, Females 47.6%) were enrolled in Phase 1a and 1b, respectively. In Phase 1a, a single dose of Q-GRFT maintained quantifiable levels in nasal passages and nasopharynx for up to 24 hours. Similarly, Q-GRFT was quantifiable in nasal and nasopharyngeal regions in the Phase 1b study. No dose accumulation effect or systemic exposure was observed. Nasal and nasopharyngeal swab eluates inhibited SARS-CoV-2 Omicron BA.5 and MERS-CoV in CPE assays. Q-GRFT did not modify olfactory sensation. No severe adverse events were reported. Thus, the nasal spray was deemed safe.
Conclusion: Intranasal Q-GRFT was safe and enhanced mucosal SARS-CoV-2 inhibitory activity in human volunteers. The results support further development of Q-GRFT as a broad-spectrum prophylactic against coronaviruses to curb ongoing infections, and for future pandemic preparedness.

Methods: K18-hACE2 mice were inoculated intranasally with 10^3 PFU of SARS-CoV-2 BA.1 Omicron (B.1.1.529). After 24 hours, mice were orally dosed q12H, as outlined in Figure 1. At 2, 3, and 4- and 6-days post-infection mice were sacrificed, and lung samples harvested. Animals were weighed and monitored daily throughout. Subsequently, viral replication in the lungs was quantified using qRT-PCR to measure total (N-gene) and sub-genomic (E-gene) viral RNA. Data were normalized to 18S for quantitation. Viral exposures measured as Areas Under viral load Curves (AUCs) were calculated by the trapezoidal method using mean values at each time-point. Separate studies in Syrian golden hamsters using individual drugs were also conducted, and total serum IgG was measured by ELISA at 4-days post-infection.

Results: Mice gained weight in all groups post-treatment, with no significant differences across all treatment groups compared to the vehicle control dosed mice (Figure 1). Coadministration of NTV with MPV displayed a trend towards lower lung viral exposure compared to the vehicle control with ~40- and ~45-fold reduction in AUC for N- and SgE-gene assays, respectively. Dosed individually, NTV and MPV reduced viral exposure 5.7- and 7.7-fold for the N-gene assay, respectively. Differences in total serum IgG concentrations were evident between vehicle and NTV (34-fold reduction, P=0.018), and MPV- (4.2-fold reduction, P=0.053) treated hamsters.

Conclusion: These data show virological efficacy of NTV and MPV against the SARS-CoV-2 BA.1 Omicron variant. The combination of NTV and MPV demonstrated a lower viral RNA exposure in the lung than either drug alone, albeit not statistically significant. Initial data indicate potential immune alterations in NTV and MPV dosed hamsters. Studies to clarify the utility of NTV/MPV combinations and further characterize the impact of antiviral therapy on IgG are warranted.

**Figure 1**. Viral exposures in K18-hACE2 mice infected with SARS-CoV-2 BA.1 Omicron (B.1.1.529), treated with NTV and MPV individually or in combination. (a) N-gene, (b) SgE-gene. n=4 per group, per time-point.
Figure: Time to 14- and 28- day mortality across the COVID-19 variant periods (adjusted Cox Proportional Hazards model)

**557 REMDESIVIR REDUCES MORTALITY IN IMMUNOCOMPROMISED PATIENTS HOSPITALIZED FOR COVID-19**

Essy Mozaffari, Aastha Chandak, Robert L. Gottlieb, Andre C. Kalil, Stephanie H. Read, Heng Jiang, Meil Chiang, Eunyoung Lee, Rikisha Gupta, Mark Thrun, Chidinma Chima-Melton

1Gilead Sciences, Inc, Foster City, CA, USA, 2Certara, Inc, New York, NY, USA, 3Baylor Scott and White Research Institute, Dallas, TX, USA, 4University of Nebraska Medical Center, Omaha, NE, USA, 5Certaon, Inc, London, United Kingdom, 6Certaon, Inc, Paris, France, 7Gilead Sciences, Inc, Taipei City, Taiwan (Republic of China), 8University of California Los Angeles, Tornace, CA, USA

**Background:** There is limited information on effectiveness of COVID-19 therapies in immunocompromised patients, who are at highest risk of hospitalizations, complications, and mortality due to COVID-19. We examined hospital all-cause mortality for early RDV use vs. no RDV use among immunocompromised COVID-19 patients across several distinct dominant variants of concern (VOC) periods: pre-Delta (Dec'20-Apr'21), Delta (May-Nov'21) and Omicron (Dec'21-Apr'22).

**Methods:** Using the Premier Healthcare Database, we identified adults with an immunocompromised condition (cancer, solid organ and hematopoietic stem cell transplant, hematologic malignancies, primary immunodeficiencies, asplenia, bone marrow failure/aplastic anemia, severe combined immunodeficiencies or HIV), hospitalized with a primary diagnosis of COVID-19. Patients treated with RDV in first 2 days of admission vs. those not treated with RDV during the hospitalization were matched using 1:1 preferential within-hospital propensity matching with replacement. Patients were excluded if discharged within 3 days of RDV initiation. Cox Proportional Hazards Model was used to examine time to 14- and 28-day mortality.

**Results:** Overall (Dec'20-Apr'22), 14,169 RDV-treated patients were matched to 5,341 unique non-RDV patients. Post-matching balance was achieved with 59% being 65+ years, 40.5% with no supplementary oxygen charges, 39% received low-flow oxygen, 19% on high-flow oxygen/non-invasive ventilation and 1.5% on invasive mechanical ventilation/ECMO at baseline. During the study period, unadjusted mortality rate was significantly lower for RDV patients at 14 days (11% [95% CI: 11%-12%] vs 15% [15%-16%]; p<.0001) and 28 days (18% [17%-18%] vs 22% [22%-23%]; p<.0001) as compared to patients that did not receive RDV. After adjusting for baseline and clinical covariates, 14-day results showed that RDV had significantly lower mortality risk compared to non-RDV across all VOC periods (overall 30% lower risk, pre-Delta 41%, Delta 23%, Omicron 25%); Similarly, 28-day results showed that RDV had significantly lower mortality risk compared to non-RDV across all VOC periods (overall 25%, pre-Delta 35%, Delta 21%, Omicron 16%) (Fig).

**Conclusion:** Timely initiation of RDV in first two days of hospital admission demonstrated significant mortality reduction in immunocompromised patients hospitalized with primary diagnosis of COVID-19. RDV demonstrated consistent benefit in an immunocompromised cohort across all variant periods of the pandemic.

Figure: Time to 14- and 28- day mortality in immunocompromised patients across the COVID-19 variant periods (adjusted Cox Proportional Hazards model)

**558 REMDESIVIR IS ASSOCIATED WITH REDUCED READMISSION AFTER COVID-19 HOSPITALIZATION**

Essy Mozaffari, Aastha Chandak, Robert L. Gottlieb, Andre C. Kalil, Vishnudas Sarda, Celine Der-Torossian, Thomas Oppelt, Lauren Dau, Mark Berry, Chidinma Chima-Melton

1Gilead Sciences, Inc, Foster City, CA, USA, 2Certara, Inc, New York, NY, USA, 3Baylor Scott and White Research Institute, Dallas, TX, USA, 4University of Nebraska Medical Center, Omaha, NE, USA, 5Certaon, Inc, London, United Kingdom, 6Certaon, Inc, Paris, France, 7Gilead Sciences, Inc, Taipei City, Taiwan (Republic of China), 8University of California Los Angeles, Tornace, CA, USA

**Background:** There is limited data on the association between COVID-19 therapy and hospital readmissions, including during evolution of the pandemic over time. We examine all cause 30-day readmissions after a COVID-19 hospitalization among remdesivir (RDV)-treated vs non-RDV treated patients across different dominant variants of concern (VOC) periods: pre-Delta (May'20-Apr'21), Delta (May-Nov'21) and Omicron (Dec'21-Apr'22).

**Methods:** Using the Premier Healthcare Database, we examined adults hospitalized with a primary diagnosis of COVID-19 (ICD-10:U07.1) who were discharged alive from the COVID-19 hospitalization. All-cause readmission to the same hospital was examined using multivariate logistic regression. The model adjusted for: age, corticosteroids use, VOC period, Charlson comorbidity index, maximum oxygenation requirements and ICU admission during COVID-19 hospitalization.

**Results:** In the study period (May'20-Apr'22), 440,601 patients with a primary diagnosis of COVID-19 were discharged alive, of which 53% received RDV. As compared to non-RDV, RDV patients were younger (median[IQR]: 62[51–73] vs 64[52–76]), with a lower proportion with supplementary oxygen charges (30% vs 52%), a higher proportion with low-flow oxygen (46% vs 36%), high-flow oxygen/non-invasive ventilation (20% vs 10%), and invasive mechanical ventilation/ECMO (4% vs 2%). Among RDV-treated, the all-cause 30-day readmission was 6.3% compared to 9.1% (p<.0001) in non-RDV treated. Lower readmission for RDV vs non-RDV was observed in Pre-delta (6.3% vs 9.3%; p<.0001), Delta (5.3% vs 7.8%; p<.0001) and Omicron (0.7% vs 9.9%; p<.0001) (Fig).

After adjusting for age and characteristics at index hospitalization including corticosteroid, RDV patients had significantly lower likelihood of all-cause 30-day readmission (OR[95% CI]:0.73[0.72-0.75]) as compared to non-RDV. Significantly Lower odds of 30-day readmission for RDV vs non-RDV patients were observed in Pre-delta (0.69[0.67-0.71]), Delta (0.72[0.68-0.76]) and Omicron- (0.87[0.83-0.92]) (Fig). Similarly, RDV-related reduction in readmissions was also seen for COVID-19 related readmissions.

**Conclusion:** RDV use during the COVID-19 hospitalization was associated with significantly lower likelihood of all-cause 30-day readmission across the VOC periods of the pandemic May 2020 till April 2022. The lower rate of hospital re-admission for RDV-treated patients was observed despite the RDV group having higher supplementary oxygen requirement during their index COVID-19 hospitalization.

Figure: All-cause 30-day re-admission for hospitalized COVID-19 patients across the variant periods

<table>
<thead>
<tr>
<th>Patients with 30-day readmissions/ number of patients, (N=15,295)</th>
<th>OR (95% CI)</th>
<th>P value</th>
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<td>Overall cohort 15,295/ (15,262)</td>
<td>0.73 (0.71 - 0.75)</td>
<td>&lt;0.001</td>
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<td>Pre-Delta 7,795/ (7,794)</td>
<td>0.76 (0.73 - 0.79)</td>
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<tr>
<td>Delta 4,293/ (4,291)</td>
<td>0.80 (0.77 - 0.83)</td>
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Figure: 30-day all-cause readmission among hospitalized COVID-19 patients across the variant periods

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## Notes
- All analyses used time from date of index hospitalization as the exposure time, including readmissions. Mortality up to 28 days was included in the analysis. Cox proportional hazards model was used for all analyses.
RISK OF DEATH IN REMDESIVIR TREATED AND UNTRATED PATIENTS WITH COVID-19 INFECTION

Adeel Butt1, Victor Talisa2, Peng Yan3, Obaid Shaikh1, Florian G. Mayr4, Weil Cornell Medicine, Pittsburgh, PA, USA, 1University of Pittsburgh, Pittsburgh, PA, USA, 1VA Pittsburgh Healthcare System, Pittsburgh, PA, USA

Background: The role of remdesivir in hospitalized patients with COVID-19 is not clear. Some studies have demonstrated improved clinical outcomes and reduced mortality, while others have failed to show a benefit.

Methods: We used the Department of Veterans Affairs’ (VA) national COVID-19 Shared Data Resource database to identify confirmed SARS-CoV-2 infected Veterans between July 1, 2020 and December 31, 2021 who were hospitalized and received remdesivir and propensity-score matched controls who had not received remdesivir. Variables for propensity-score matching included demographics, comorbidities, time and location of diagnosis/admission, severity of illness, and use of other potential COVID-19 therapeutics. Primary outcome of interest was 28-day mortality in the entire matched cohort, and among subgroups stratified by use of supplemental oxygen.

Results: Among 238,298 SARS-CoV-2 infected Veterans, 31,632 were hospitalized, and 13,147 received remdesivir. Our final dataset included 3,583 remdesivir recipients and 3,583 propensity-score matched controls. Probability of survival at 28 days of all admissions was higher in those who had received remdesivir (P=0.032). Remdesivir recipients had better survival among the group who received supplemental oxygen but did not require mechanical ventilation (P=0.005).

Conclusion: Remdesivir demonstrated a survival benefit among hospitalized patients with COVID-19 which was limited to those who received supplemental oxygen but did not require mechanical ventilation.

PROBABILITY OF EVENT-FREE SURVIVAL AMONG REMDESIVIR TREATED PERSONS (N=3,583) AND PROPENSITY-SCORE MATCHED UNTREATED PERSONS (N=3,583), OVERALL AND STRATIFIED BY OXYGEN REQUIREMENT AT ADMISSION.

Table 1: Summary of findings and certainty of evidence, by respiratory support

<table>
<thead>
<tr>
<th>Respiratory Support</th>
<th>Evidence of Treatment Effect</th>
<th>Certainty of Evidence</th>
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<tbody>
<tr>
<td>No mechanical ventilation</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Low flow oxygen</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>High flow oxygen</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Non-invasive positive pressure</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>Low</td>
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Remdesivir recipients were less likely to die versus those who did not receive remdesivir. The probability of survival at 28 days was higher among those who had received remdesivir including high-flow oxygen, 253/454 (20.9%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence).

Remdesivir demonstrated a survival benefit among hospitalized patients requiring no or conventional oxygen support, but patients requiring more respiratory support may not benefit. These findings may inform clinical guidelines, especially due to increasing resistance to current monomodal antibodies.

Table 1: Summary of findings and certainty of evidence, by respiratory support

560 REMDESIVIR IN HOSPITALIZED COVID-19 PATIENTS: INDIVIDUAL PATIENT DATA META-ANALYSIS

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1University Hospital Basel, Basel, Switzerland, 2Université Paris Cité, Paris, France, 3Oslo Centre for Biostatistics & Epidemiology, Oslo University Hospital, Oslo, Norway, 4Université Sorbonne Paris Nord, Paris, France

Background: The role of remdesivir in hospitalized patients with COVID-19 is not clear. Some studies have demonstrated improved clinical outcomes and reduced mortality, while others have failed to show a benefit.

Methods: We used the Department of Veterans Affairs’ (VA) national COVID-19 Shared Data Resource database to identify confirmed SARS-CoV-2 infected Veterans between July 1, 2020 and December 31, 2021 who were hospitalized and received remdesivir and propensity-score matched controls who had not received remdesivir. Variables for propensity-score matching included demographics, comorbidities, time and location of diagnosis/admission, severity of illness, and use of other potential COVID-19 therapeutics. Primary outcome of interest was 28-day mortality in the entire matched cohort, and among subgroups stratified by use of supplemental oxygen.

Results: Among 238,298 SARS-CoV-2 infected Veterans, 31,632 were hospitalized, and 13,147 received remdesivir. Our final dataset included 3,583 remdesivir recipients and 3,583 propensity-score matched controls. Probability of survival at 28 days of all admissions was higher in those who had received remdesivir (P=0.032). Remdesivir recipients had better survival among the group who received supplemental oxygen but did not require mechanical ventilation (P=0.005).

Conclusion: Remdesivir demonstrated a survival benefit among hospitalized patients with COVID-19 which was limited to those who received supplemental oxygen but did not require mechanical ventilation.

PROBABILITY OF EVENT-FREE SURVIVAL AMONG REMDESIVIR TREATED PERSONS (N=3,583) AND PROPENSITY-SCORE MATCHED UNTREATED PERSONS (N=3,583), OVERALL AND STRATIFIED BY OXYGEN REQUIREMENT AT ADMISSION.

Table 1: Summary of findings and certainty of evidence, by respiratory support

<table>
<thead>
<tr>
<th>Respiratory Support</th>
<th>Evidence of Treatment Effect</th>
<th>Certainty of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mechanical ventilation</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Low flow oxygen</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>High flow oxygen</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Non-invasive positive pressure</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Remdesivir recipients were less likely to die versus those who did not receive remdesivir. The probability of survival at 28 days was higher among those who had received remdesivir including high-flow oxygen, 253/454 (20.9%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence). Of those receiving no or low flow oxygen, 409/4473 (9.1%) assigned to remdesivir died versus 241/846 (28.5%) assigned to no remdesivir (aOR 1.10 [0.88-1.38]; low certainty evidence).

Remdesivir demonstrated a survival benefit among hospitalized patients requiring no or conventional oxygen support, but patients requiring more respiratory support may not benefit. These findings may inform clinical guidelines, especially due to increasing resistance to current monomodal antibodies.

Table 1: Summary of findings and certainty of evidence, by respiratory support

561 REMDESIVIR RESISTANCE ANALYSES FROM THE PINETREE STUDY IN OUTPATIENTS WITH COVID-19

Lauren Rodriguez1, Hery W. Lee1, Jioni Li2, Ross Martin2, Don Han2, Simin Xu1, Gregory Camus1, Jason K. Perry1, Robert Hyland1, Danielle P. Porter1, Mazin Abdelghany1, Matthias Götte2, Charlotte Hedskog2

1Gilead Sciences, Inc, Foster City, CA, USA, 2Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

Background: Remdesivir (RDV) is a broad-spectrum nucleotide analog antiviral approved for the treatment of COVID-19 in patients who are hospitalized or non-hospitalized and at risk of progressing to severe disease. Here we present SARS-CoV-2 resistance analyses from the Phase 3 PINETREE trial.

Methods: PINETREE was a double-blind, placebo-controlled trial of non-hospitalized participants (N=562) with COVID-19 and ≥ 1 risk factor for disease progression, randomized to receive RDV or placebo once-daily for 3 days. The whole genome of SARS-CoV-2 was sequenced from nasopharyngeal swabs collected at days 1 (baseline), 2, 3, 7, and 14 using next-generation sequencing. Emergent amino acid substitutions in SARS-CoV-2 from participants treated
with RDV were tested in a replicon system to determine if they alter sensitivity to RDV.

**Results:** Resistance analysis criteria included all participants in the RDV group and 50% in the placebo group with viral load above the lower limit of detection for the viral load assay. Of 281 participants who met these criteria, baseline and postbaseline sequencing data were available for 115/130 (88.3%) participants in the RDV group and 129/151 (85.4%) participants in the placebo group. Table 1. Among these, emergent substitutions in Nsp12 were observed in 8/115 (7.0%) in the RDV group and 129/151 (8.4%) in the placebo group. A total of 7 emergent amino acid substitutions in Nsp12 were observed in the RDV group, but not in the placebo group. Among these, only one substitution from one participant (A376V; first detected at day 14), showed reduced in vitro susceptibility to RDV, with a half-maximal effective concentration (EC_{50}) fold-change of 12.6 compared with a wildtype reference. The participant achieved clinical recovery by day 14. None of the other substitutions impacted RDV susceptibility (EC_{50} fold-change ≥ 1.4). Emergent substitutions in Nsp12, Nsp10, Nsp13, or Nsp14 were detected in 10/115 (8.7%) of participants in the RDV group and 10/129 (7.8%) in the placebo group, with substitutions in the RDV group showing similar susceptibility to RDV as the wildtype reference (EC_{50} fold-change ≤ 2.3).

**Conclusion:** Overall, emergent substitutions in the SARS-CoV-2 replication complex including Nsp12 were observed with similar frequency in the RDV and placebo groups, with only one participant developing a substitution associated with reduced in vitro RDV susceptibility, indicating a high barrier to the development of RDV resistance in COVID-19 patients.

**Table 1:** Phenotypic Resistance Data

<table>
<thead>
<tr>
<th></th>
<th>RDV</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical population (%)</td>
<td>100 (46.0%)</td>
<td>100 (46.4%)</td>
</tr>
<tr>
<td>Emergent substitutions (%)</td>
<td>12/115 (10.4%)</td>
<td>13/129 (9.9%)</td>
</tr>
<tr>
<td>Nsp12 substitutions (%)</td>
<td>8/115 (7.0%)</td>
<td>10/129 (7.8%)</td>
</tr>
<tr>
<td>Participants with Nsp12 substitutions affecting RDV activity (%)</td>
<td>7/115 (6.1%)</td>
<td>9/129 (7.0%)</td>
</tr>
</tbody>
</table>

**563 SARS-CoV-2 VIRAL LOAD CHANGE IN A RANDOMIZED TRIAL ON THREE DIFFERENT EARLY THERAPIES**

Valentina Mazzotta, Simone Lanini, Giulia Matsusli, Francesca Colavita, Alessandra Vergori, Emanuela Giombini, Silvia Rosati, Sabrina Minicucci, Martina Reuca, Francesca Gavazzoni, Enrico Girardi, Emanuele Nicastrì, Francesco Vaia, Fabrizio Maggi, Andrea Antinori

**Background:** SARS-CoV-2 Omicron sublineages exhibit evolving escape to in vitro neutralization by monoclonal antibodies (mAbs), with an unclear impact on in vivo treatment response.

Our aim is to assess the impact of SARS-CoV-2 variants on the decline of viral load (VL) after treatment with 3 different drugs approved in EU for the early treatment of patients with mild/moderate COVID-19.

**Methods:** Post-hoc analysis from MONET (EudraCT: 2021-004188-28), phase 4 open-label RCT to assess efficacy of 500 mg intravenous tixagivab/igilavir (TIX/CIL) and oral 5-days course of NMV/1/300/100 mg BD, in non-hospitalized high-risk patients (pts) with early COVID-19. Pts’ features were analyzed as binary variables by Chi-squared test. SARS-CoV-2 VL in nasopharyngeal swabs was carried out at randomization (1d) and at day 7 (7d) by cycle threshold value (Ct). Variant sequencing was performed at 1d. Ct variation was assessed by mixed effect log-linear model including random intercept at pts’ level, log of Ct as independent variable, time, arm, viral variant as dependent variables, and interaction between time and arm. Multiple comparisons were adjusted by Bonferroni.

**Results:** Among the 320 pts included between 4 Mar and 16 Nov, 2022, 108 (33.75%) received NMV/t, 103 (32.19%) TIX/CIL, and 109 (34.06%) CIL. Main characteristics were balanced across arms.

Most of the pts were infected either with BA.2 (N=194; 60.63%) or BA.4/BA.5 (N=100; 31.25%) (Fig1A). VL at 1d was similar across the arms. In contrast, mean 7d VL was significantly lower in pts receiving NMV/t than in those receiving TIX/CIL or SOT (P< 0.001). No significant VL variation was observed between the mAb arms (Fig1B). The analysis of the impact of viral variants suggests that while VL was significantly affected by variants (P<0.034), the superior effect of NMV/1 over mAbs was homogeneous across all variant groups (P=0.290 for interaction) (Fig1C).

**Conclusion:** Our study provides for the first time strong in vivo evidence that, when used against Omicron lineages, NMV/1 exerts a stronger antiviral effect than mAbs. These results confirm previous in vitro evidence suggesting that mAbs may not retain neutralizing activity against all Omicron sublineages and provide preliminary information on how to use VL variation as a surrogate marker of efficacy. Further studies are needed to investigate whether the superior virologic activity of NMV/1 over mAbs is confirmed for newly emerging variants, including BQ.1.1 or XBB.
A RANDOMIZED TRIAL ON EARLY THERAPY IN COVID-19 HIGH-RISK OUTPATIENTS IN OMICRON ERA
Valentina Mazzotta1, Simone Lanini1, Lavinia Fabeni1, Alessandra Vergori1, Alessandra D’Abramo1, Maria Mazzadra Plazzi1, Lia Cortellini1, Samir Al Moghazi1, Emanuele Nicastri1, Francesco Vaia1, Fabrizio Maggi1, Andrea Antinori1
1National Institute for Infectious Diseases L.Spallanzani, IRCCS, Rome, Italy, 2University College London, London, London, United Kingdom

Background: Antivirals and monoclonal antibodies (mAbs) were approved for early treatment of COVID-19 based on data from trials conducted in unvaccinated people before the Omicron era. The comparative effectiveness of different treatments is unknown. We present the results of the interim analysis of MONET trial (EudraCT: 2021-004188-28).

Methods: In this ongoing multicenter, open-label, phase 4 trial, we randomly assigned, in a 1:1:1 ratio, non-hospitalized patients with early symptomatic Covid-19 (≤5 days after symptoms onset) and ≥1 risk factor for disease progression, to receive 500 mg of intravenous sotrovimab (SOT) or 600 mg of intramuscular tixagevimab/cilgavimab (TIX/CIL) or oral 5-days course of nirmatrelvir/ritonavir (NMV/r). Primary outcome was hospitalization or death for any cause within 29 days after randomization, reported as cumulative incidence per 100 patients (pts) (3/5 immunosuppressed) had disease progression leading to hospitalization [1.25% (95% CI 0.4%-2.89%)], in 1 SOT (0.75%, 95% CI 0.01%-4.1%), 4 in TIX/CIL (3.08%, 95% CI 0.84%-7.69%) and none in NMV/r arm (P=0.030). No deaths or ICU admissions were observed. Among the hospitalized pts, 3 were infected with BA.2 (1 SOT, 2 TIX/CIL), one with BA.4/5, and one BQ.1.1 (both TIX/CIL). No serious adverse events and no kidney or liver toxicity were reported. Temporal trend of inflammation markers was similar in the three arms, and their estimates are shown in Fig.1B. Kinetics of antibody was reported in Fig.1C. The plot shows a rapid increase of anti-S in both mAb arm and a linear increase of IgG in the NMV/r arm. Anti-N IgG kinetics was similar in the three arms.

Conclusion: By these data the overall cumulative risk of clinical failure in mild Covid-19 occurring in the Omicron era is low. The hypothesis that differences in clinical progression among the three arms could be related to different activity against the Omicron variant subvariant observed in vitro should be further investigated. Type of treatment does not seem to influence the development of the natural antibody response.

565 SARS-CoV-2 OMICRON VIRAL LOAD DECREASE AFTER MONOCLONAL ANTIBODIES OR ANTIVIRALS
Valentina Mazzotta1, Alessandro Cozzi Lepri2, Francesca Colavita1, Simone Lanini1, Giulia Matsus4, Gaetano Maffongelli1, Alessandra Vergori1, Eleonora Lalle1, Jessica Paulicelli1, Pierluca Piselli1, Enrico Girardi1, Francesco Vaia1, Emanuele Nicastri1, Francesco Vaia1, Fabrizio Maggi1, Andrea Antinori1
1National Institute for Infectious Diseases L.Spallanzani, IRCCS, Rome, Italy, 2University College London, London, London, United Kingdom

Background: Sotrovimab (SOT) and Tixagevimab/cilgavimab (TIX/CIL) were approved on the basis of clinical trials conducted in the pre-Omicron era. It is unknown whether they are still effective in the current SARS-CoV-2 pandemic. This study compared the viral load decrease observed with SOT and TIX/CIL in comparison with the historical control population. We investigated whether the viral load decrease was influenced by the severity of the disease, the variant of SARS-CoV-2 and the type of antiviral or monoclonal antibody used.

Methods: Single-center observational comparison study enrolling all consecutive patients (pts) seen for care at a confirmed SARS-CoV-2 Omicron diagnosis and who met the AIFA criteria for eligibility for treatment with RDV, MLN, NMV/r, TIX/CIL, or SOT. Treatment allocation was subject to drug availability, time from symptoms onset, and comorbidities. Nasopharyngeal
566 INCIDENCE AND PREDICTORS OF CLINICAL PROGRESSION IN AN EARLY TREATED COVID-19 COHORT

Valentina Mazzotta1, Alessandro Cozzi Lepri2, Simone Lanini1, Silvia Meschi1, Alessandra Vergeri1, Tommaso Ascoli Bartoli1, Giulia Gramigna1, Giuseppe Giannico1, Alessandra D’Abramo1, Claudia Cimaglia1, Enrico Girardi1, Francesco Vaia1, Fabrizio Maggi1, Emanuele Nicastri1, Andrea Antinori1

INMI COVID-19 Outpatients Treatment Study Group
National Institute for Infectious Diseases L.Spolanzani, INHS, Rome, Italy; 1University College London, London, United Kingdom

Background: Early treatment for preventing severe outcome of COVID-19 in high-risk not-hospitalized patients (pts) by monoclonal antibodies or antivirals represented a high-priority approach. Real-world evidence (RWE) from observational studies could provide information on clinical effectiveness and predictors of treatment failure.

Methods: Single-center observational study on SARS-CoV-2 pts, not requiring hospital admission but having high-risk of severe outcome from COVID-19. All were selected for early treatment with monoclonal antibodies or antivirals from March 2021 to November 2022. Participants were classified according to whether they were hospitalized due to severe COVID-19 or died by day 30 from starting treatment in the outpatient setting (baseline). We conducted a logistic regression analysis with this binary endpoint and 4 main exposures of interest measured at baseline: i) age (>75 years old) ii) vaccination status iii) VoC and iv) immunosuppression or having received immunosuppressive therapy. We built a separate model for each of these exposures, which included a specific set of potential confounders.

Results: 3,491 pts, female 48.6%, median age 67 yrs (IQR 55-77), fully vaccinated 83.7%; previous infection 4.6%; CVD 52.2%; cancer 24.6%; immunodeficiency 46.0%. Prevalence of SARS-CoV-2 VoC: delta 8.7%, BA.1 16.9%, BA.2 6.8%, BA.4/5 12.2%, BQ.1 0.1%, other 3.0% (Tab.1A). Treatment exposure was BAM/ETE 569 (16.5%), CAS/IMD 262 (7.6%), DOT 935 (27.1%), TIX/CIL 79 (2.3%), NMV/r 555 (16.1%), MLN 684 (19.8%), RDV 356 (10.3%). Primary endpoint occurred in 80/3,491 pts with a day-30 incident risk of 2.3% (95%CI 1.8-2.9). Tab.1B shows the unadjusted and adjusted odds ratios (OR) of hospitalization due to COVID-19 or death by day 30. After controlling for potential confounders, higher risk was observed for the unvaccinated (OR 1.95;95%CI 1.03-3.71) and for those affected by immunodeficiency (1.73; 1.04-2.89). Having delta as reference variant, an increased risk was observed for BA.2 (2.08; 1.00-2.34). No evidence for a difference vs. TIX/CIL was seen in those infected with BA.2 (p=0.05) (Fig.1 C-D).

Conclusion: In this RWE study, largely represented by vaccinated people and prevaletnly observed in the Omicron era, the estimated risk of clinical failure of early treatment was slightly higher than that recorded in the experimental arms of randomized studies. The analysis confirms that among those eligible for early treatment, the unvaccinated and those with severe immunodeficiency are at higher risk of developing severe COVID-19.
Uptake of nirmatrelvir-ritonavir (NMV/r) increased over time coinciding with national COVID-19 vaccine booster uptake remaining low and preventable COVID-19 deaths continue to occur, making access to oral antivirals for those most at risk of severe COVID-19 outcomes essential. Methods: We estimated age and gender adjusted prevalence ratios of nirmatrelvir-ritonavir (NMV/r) uptake by sociodemographics, clinical characteristics, and prescription eligibility (based on age, underlying medical conditions, body mass index, physical inactivity, pregnancy, or smokers), among participants in a large U.S. national prospective cohort who were infected with SARS-CoV-2 between December 2021 and October 2022. Among participants who reported NMV/r uptake, we also described the proportion who reported (1) taking NMV/r as directed and (2) NMV/r was helpful for reducing COVID-19 symptoms. Results: Among 1,594 participants with a SARS-CoV-2 infection as of October 2022, 1,356 were eligible for NMV/r prescription; of whom 209 (15.4% [95%CI:13.0–17.3]) reported receiving NMV/r. NMV/r uptake increased from 2.2% (95%CI:1.0–3.4) between December 2021 and March 2022 to 16.5% (95%CI:13.5–17.3) between April and July 2022 and 28.6% (95%CI:24.4–32.8) between August and October 2022. Participants ≥65 years of age reported the highest uptake of NMV/r (30.2% [95%CI:22.2–38.2]) compared to younger age groups. Black non-Hispanic participants (7.2% [95%CI:2.4–12.0]) and those in the lowest income group (10.6% [95%CI:7.3–13.8]) had lower uptake than white non-Hispanic (15.8% [95%CI:13.6–18.0]) and high-income individuals (18.4% [95%CI:15.2–21.7]), respectively. Participants with type 2 diabetes had greater uptake (28.8% [95%CI:20.4–37.3]) compared to those without it (12.4% [95%CI:4.8–20.0]). Among a subset of 278 participants who had a prior SARS-CoV-2 infection, those who had a history of long COVID reported greater uptake (22.0% [95%CI:13.9–30.1]) for a subsequent SARS-CoV-2 infection than those without a history of long COVID (7.9% [95%CI:3.9–11.8]). Among all participants who were prescribed NMV/r (N=216), 89% (95%CI:85–93) stated NMV/r was helpful for reducing COVID-19 symptoms. Conclusion: Uptake of NMV/r increased over time coinciding with national efforts to increase awareness and access. However, most individuals who were eligible for NMV/r did not receive it. Lower NMV/r uptake among racial/ethnic minorities and individuals with lower household income suggests a need to improve awareness and address barriers to uptake in these populations. Adjusted Prevalence Ratios of Nirmatrelvir-Ritonavir (NMV/r) Uptake by Sociodemographic and Clinical Factors – United States CHASING COVID Cohort Study Participants with SARS-CoV-2 Infection, December 2021–October 2022

Background: A 5-day course of nirmatrelvir-ritonavir (N/R) can significantly reduce the hospitalization and death rates and the duration of infectiousness in high-risk SARS-CoV-2 patients. However, in a fraction of treated individuals virus rebounds following an initial recovery after treatment. The mechanism driving rebound is not well understood. We hypothesize that treatment with N/R near the time of symptom onset halts the depletion of target cells, but does not fully eliminate the virus, and thus can lead to viral rebound. Methods: Previously, we and others have developed viral dynamic models and successfully used them to fit data on SARS-CoV-2 infection. Here we expand these models and incorporate N/R pharmacokinetic and pharmacodynamic effects and an adaptive immune response. Results: We fit this model to the data presented in Charness et al., NEJM (2022) where longitudinal quantitative PCR data is available for 3 individuals who experienced viral rebounds after taking N/R. We found that the model fit the data well. By varying model parameters from their best-fit values, we show the occurrence of viral rebound is sensitive to model parameters, and the time treatment is initiated, which may explain why only a fraction of individuals rebound. Finally, the model with its best-fit parameter values was used to test the therapeutic effects of treatment extended to 10 days or a second 5-day course of N/R initiated one day after symptoms recur. Conclusion: Our model fits predicted that virus is not fully eliminated during N/R treatment and supported our initial hypothesis that at the end of treatment target cells are available to allow viral resurgence. Simulating the effect of starting treatment later, we find the probability of viral rebound occurring decreases, suggesting that delaying treatment may be a strategy to reduce viral rebound. However, N/R treatment accelerates viral clearance and hence potentially can reduce viral transmission. Thus, delaying treatment may have a detrimental effect on public health and could also have impact on the severity of disease in the high-risk patients for whom N/R is recommended. Increasing treatment from 5 to 10 days continues to preserve target cells and thus may still allow viral rebound if viable virus is present at the end of treatment and sufficient adaptive immunity has not developed. Simulating giving a second course of treatment one day after symptoms reappear, did not prevent rebound. Nirmatrelvir Use and Hospitalizations or Death in Individuals with COVID-19

<table>
<thead>
<tr>
<th>Age group</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15 yrs</td>
<td>1.05 (0.56, 1.90)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>15 to 64 yrs</td>
<td>2.73 (1.58, 4.73)</td>
<td>0.39 (0.15, 0.98)</td>
<td>0.98 (0.50, 1.83)</td>
</tr>
<tr>
<td>≥ 65 yrs</td>
<td>0.05 (0.01, 0.28)</td>
<td>1.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Adjusted for age and gender

Background: Nirmatrelvir-ritonavir (NMV/r) was granted Emergency Use Authorization in December 2021 for treatment of early symptomatic patients at high-risk SARS-CoV-2 patients. However, in a fraction of treated individuals virus rebounds following an initial recovery after treatment. The mechanism driving rebound is not well understood. We hypothesize that treatment with N/R near the time of symptom onset halts the depletion of target cells, but does not fully eliminate the virus, and thus can lead to viral rebound. Methods: Previously, we and others have developed viral dynamic models and successfully used them to fit data on SARS-CoV-2 infection. Here we expand these models and incorporate N/R pharmacokinetic and pharmacodynamic effects and an adaptive immune response. Results: We fit this model to the data presented in Charness et al., NEJM (2022) where longitudinal quantitative PCR data is available for 3 individuals who experienced viral rebounds after taking N/R. We found that the model fit the data well. By varying model parameters from their best-fit values, we show the occurrence of viral rebound is sensitive to model parameters, and the time treatment is initiated, which may explain why only a fraction of individuals rebound. Finally, the model with its best-fit parameter values was used to test the therapeutic effects of treatment extended to 10 days or a second 5-day course of N/R initiated one day after symptoms recur. Conclusion: Our model fits predicted that virus is not fully eliminated during N/R treatment and supported our initial hypothesis that at the end of treatment target cells are available to allow viral resurgence. Simulating the effect of starting treatment later, we find the probability of viral rebound occurring decreases, suggesting that delaying treatment may be a strategy to reduce viral rebound. However, N/R treatment accelerates viral clearance and hence potentially can reduce viral transmission. Thus, delaying treatment may have a detrimental effect on public health and could also have impact on the severity of disease in the high-risk patients for whom N/R is recommended. Increasing treatment from 5 to 10 days continues to preserve target cells and thus may still allow viral rebound if viable virus is present at the end of treatment and sufficient adaptive immunity has not developed. Simulating giving a second course of treatment one day after symptoms reappear, did not prevent rebound. Background: A 5-day course of nirmatrelvir-ritonavir (N/R) can significantly reduce the hospitalization and death rates and the duration of infectiousness in high-risk SARS-CoV-2 patients. However, in a fraction of treated individuals virus rebounds following an initial recovery after treatment. The mechanism driving rebound is not well understood. We hypothesize that treatment with N/R near the time of symptom onset halts the depletion of target cells, but does not fully eliminate the virus, and thus can lead to viral rebound. Methods: Previously, we and others have developed viral dynamic models and successfully used them to fit data on SARS-CoV-2 infection. Here we expand these models and incorporate N/R pharmacokinetic and pharmacodynamic effects and an adaptive immune response. Results: We fit this model to the data presented in Charness et al., NEJM (2022) where longitudinal quantitative PCR data is available for 3 individuals who experienced viral rebounds after taking N/R. We found that the model fit the data well. By varying model parameters from their best-fit values, we show the occurrence of viral rebound is sensitive to model parameters, and the time treatment is initiated, which may explain why only a fraction of individuals rebound. Finally, the model with its best-fit parameter values was used to test the therapeutic effects of treatment extended to 10 days or a second 5-day course of N/R initiated one day after symptoms recur. Conclusion: Our model fits predicted that virus is not fully eliminated during N/R treatment and supported our initial hypothesis that at the end of treatment target cells are available to allow viral resurgence. Simulating the effect of starting treatment later, we find the probability of viral rebound occurring decreases, suggesting that delaying treatment may be a strategy to reduce viral rebound. However, N/R treatment accelerates viral clearance and hence potentially can reduce viral transmission. Thus, delaying treatment may have a detrimental effect on public health and could also have impact on the severity of disease in the high-risk patients for whom N/R is recommended. Increasing treatment from 5 to 10 days continues to preserve target cells and thus may still allow viral rebound if viable virus is present at the end of treatment and sufficient adaptive immunity has not developed. Simulating giving a second course of treatment one day after symptoms reappear, did not prevent rebound. Background: Nirmatrelvir-ritonavir (NMV/r) was granted Emergency Use Authorization in December 2021 for treatment of early symptomatic patients with mild to moderate COVID-19 at high risk of progression. However, its benefit is specific population subgroups remains unclear. Methods: We used a matched cohort design to evaluate a target trial within the VA COVID-19 Shared Data Resource database. Eligible individuals were those with at least two episodes of care in the VA in the last 2 years, who had a first confirmed SARS-CoV-2 infection between January 1 and August 31, 2022 and were free of hospitalization or death within 3 days of testing positive. Those hospitalized in the previous 60-days and those who received Molnupiravir after diagnosis were ineligible. Among the eligible individuals, we matched those prescribed NMV/r with those not prescribed NMV/r within 3 days of diagnosis. Controls were matched 1:1 on age (5-year blocks), race, sex, body mass index, Charlson Comorbidity Index, VA facility where NMV/r was prescribed, and…
570 MOLNUPAVIR IN PREVIOUSLY UNINFECTED NONHOSPITALIZED PERSONS WITH COVID-19

Adeel Butt¹, Peng Yan¹, Obaid Shaikh¹, Saad Omer¹, Florian G. Mayr¹, Victor Talisa¹

¹Well Cornell Medicine, Pittsburgh, PA, USA; VA Pittsburgh Healthcare System, Pittsburgh, PA, USA; Yale University, New Haven, CT, USA; University of Pittsburgh, Pittsburgh, PA, USA

Background: Clinical benefit of molnupiravir (MPV) in COVID-19 infected subgroups is unclear.

Methods: We used a matched cohort design emulating a target trial to analyze the VA COVID-19 Shared Resource database to determine the association of MPV with hospitalization or death within 30 days with untreated controls in previously uninfected non-hospitalized persons. Incidence of hospitalization/death and absolute risk difference (ARD) with 95% confidence intervals were calculated for the treated and untreated groups.

Results: Among 1,459 matched pairs, the incidence of hospitalization/death was not different among MPV treated vs. untreated controls (48 vs. 44 cases; ARD 95% CI 0.07 [-0.94, 1.49]). No benefit was observed among those >60 or <60 years old (ARD 0.27 [-1.25, 1.79] vs. -0.29 [-1.22, 1.80]), those with specific comorbidities, or by vaccination status. A significant benefit was observed in asymptomatic but not in symptomatic persons (ARD -2.80 [-4.74, -0.87] vs. -1.2 [-0.31, 2.55]). Kaplan-Meier curves did not show a significant reduction in proportion of persons who were hospitalized or died among those treated with MPV compared with untreated controls (logrank P=0.7).

Conclusion: MPV was not associated with a significant reduction in hospitalization or death within 30 days of COVID-19 diagnosis overall. A subgroup of patients presenting without symptoms experienced a benefit. INCIDENCE OF HOSPITALIZATION OR DEATH WITHIN 30 DAYS AND ABSOLUTE RISK DIFFERENCE AMONG PATIENTS WHO RECEIVED MOLNUPAVIR AND CONTROLS.

RESULTS FOR THE MATCHED GROUPS.

Figure. Incidence of hospitalization or death within 30 days and absolute risk difference among patients who received (Molnupiravir) and controls. Results for the matched groups.

571 TIXAGEVIMAB/CILGAVIMAB IM AND IV FOR COVID-19: A RANDOMIZED CONTROLLED ACTIV-2 TRIAL

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¹ACTIV-2/A5401 Study Team

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Background: Within the ACTIV-2/A5401 platform (NCT04518410), the safety and efficacy of tixagevimab/cilgavimab (T/C), an anti-SARS-CoV-2 monoclonal antibody combination, was studied in outpatients with COVID-19. Intravenous (IV) and intramuscular (IM) administration of T/C were assessed.

Methods: Non-hospitalized adults ≥18 years enrolled within 10 days of positive SARS-CoV-2 test and symptom onset. Participants at higher risk of disease progression were eligible for IV T/C 300mg (150mg each component) or placebo; all were eligible for IM T/C 600mg (300mg each) administered to the lateral thigh or placebo. Co-primary outcomes were: time to symptom improvement through day 28; nasopharyngeal (NP) SARS-CoV-2 RNA below lower limit of quantification (LLoQ) on days 3, 7, and 14; and treatment emergent adverse events.

Results: Between February and May 2021, 233 participants (106 T/C, 127 placebo) initiated study intervention and were included in the IM analysis and 114 participants (58 T/C, 56 placebo) in the IV analysis; the IV study was stopped early for administrative reasons. Both studies enrolled 45% Latinx; the IM and IV populations included 12% and 19% Black participants, 49% and 59% female sex at birth, and median age was 39 and 44 years, respectively, all of which were balanced between active vs placebo for each. Median (IQR) days from symptom onset at enrollment was 6 (4, 7). There were no differences in time to symptom improvement comparing IM T/C to placebo (median 8 [IQR 7, 12] vs 10 [8, 13] days; p=0.35) or IV T/C to placebo (11 [9, 15] vs 10 [7, 15] days; p=0.71). A significantly greater proportion (80%) in the IM T/C arm had NP SARS-CoV-2 RNA below LLoQ at day 7 compared to placebo (65%), but not days...
A RANOMIZED TRIAL OF IVERMECTIN 600 MCG/KG VS PLACEBO IN MILD/ MODERATE COVID-19

Susanna Naggie 1, David R. Boulware 2, Christopher J. Lindell 3, Thomas G. Stewart 4, G. Michael Felker 5, Matthew W. McCarthy 6, Russell L. Rothman 3, Sybil Wilson 7, Allison Delong 7, Sean Collins 8, Sarah E. Dunsmore 9, Stacey J. Adami 9, Florence Thicklin 10, Elizabeth Shenkman 11, Adrian F. Hernandez 5, Susanna Naggie 1

Accelerating Covid-19 Therapeutic Interventions and Vaccines (ACTIV) - 6 Study Group and Investigators

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Background: Whether ivermectin, with a maximum targeted dose of 600 µg/kg, shortens symptom duration or prevents hospitalization among outpatients with mild to moderate coronavirus disease 2019 (COVID-19) remains unknown. Our objective was to evaluate the effectiveness of ivermectin, maximum targeted dose of 600 µg/kg, daily for 6 days compared with placebo for the treatment of early mild to moderate COVID-19.

Methods: ACTIV-6, an ongoing, decentralized, randomized, double-blind, placebo-controlled, platform trial, was designed to evaluate repurposed therapies in outpatients with mild to moderate COVID-19. A total of 1206 participants aged ≥30 years with confirmed COVID-19, experiencing ≥2 experiences in outpatients with mild to moderate COVID-19. A total of 1206 participants aged ≥30 years with confirmed COVID-19, experiencing ≥2

Results: Among 1206 randomized participants who received study medication or placebo, median (interquartile range) age was 48 (38–58) years; 713 (59%) were women; and 1008 (84%) reported ≥2 SARS-CoV-2 vaccine doses. Median time to recovery was 11 (11–12) days in the ivermectin group and 11 (11–12) days in the placebo group. The hazard ratio (HR) (95% credible interval [CrI]) posterior probability of benefit) for improvement in time to recovery was 1.02 (0.92–1.13; P[HRI]>1|=0.68). In those receiving ivermectin, 34 (5.7%) were hospitalized, died, or had urgent or emergency care visits compared with 36 (6.0%) receiving placebo (HR 1.0, 0.6–1.5; P[HRI]<|=0.53). In the ivermectin group, 1 participant died and 4 were hospitalized (0.8%); 2 participants (0.3%) were hospitalized in the placebo group and there were no deaths. Adverse events were uncommon in both groups.

Conclusion: Among outpatients with mild to moderate COVID-19, treatment with ivermectin, with a maximum targeted dose of 600 µg/kg daily for 6 days, compared with placebo did not improve time to recovery. These findings do not support the use of ivermectin in patients with mild to moderate COVID-19.

Primary and secondary outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ivermectin (placebo)</th>
<th>Placebo (placebo)</th>
<th>Adjusted estimate (95% CrI)</th>
<th>Treatment Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary endpoint, time to recovery*</td>
<td>11 (11–12) days</td>
<td>11 (11–12) days</td>
<td>1.0 (0.6–1.5)</td>
<td>0.53</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery at 28 days</td>
<td>8 (6–9) days</td>
<td>8 (6–9) days</td>
<td>1.0 (0.6–1.5)</td>
<td>0.53</td>
</tr>
<tr>
<td>Hospitalization, urgent care, death</td>
<td>34 (31–37)</td>
<td>36 (33–39)</td>
<td>0.9 (0.6–1.3)</td>
<td>0.52</td>
</tr>
<tr>
<td>Time to symptom improvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevation of C-reactive protein</td>
<td>30 (27–32) mg/L</td>
<td>30 (27–32) mg/L</td>
<td>1.0 (0.8–1.3)</td>
<td>0.51</td>
</tr>
<tr>
<td>Clinical diagnoses related to outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondarily, median (IQR)</td>
<td>11 (11–12) days</td>
<td>11 (11–12) days</td>
<td>1.0 (0.6–1.5)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

* Secondary analysis limited to those who received a full course of study therapy (6 days). AEs, adverse events; CrI, credible interval; HR, hazard ratio; placebo, median (interquartile range) age was 48 (38–58) years; 713 (59%) were women; and 1008 (84%) reported ≥2 SARS-CoV-2 vaccine doses. Median time to recovery was 11 (11–12) days in the ivermectin group and 11 (11–12) days in the placebo group. The hazard ratio (HR) (95% credible interval [CrI]) posterior probability of benefit) for improvement in time to recovery was 1.02 (0.92–1.13; P[HRI]>1|=0.68). In those receiving ivermectin, 34 (5.7%) were hospitalized, died, or had urgent or emergency care visits compared with 36 (6.0%) receiving placebo (HR 1.0, 0.6–1.5; P[HRI]<|=0.53). In the ivermectin group, 1 participant died and 4 were hospitalized (0.8%); 2 participants (0.3%) were hospitalized in the placebo group and there were no deaths. Adverse events were uncommon in both groups.

Conclusion: Among outpatients with mild to moderate COVID-19, treatment with ivermectin, with a maximum targeted dose of 600 µg/kg daily for 6 days, compared with placebo did not improve time to recovery. These findings do not support the use of ivermectin in patients with mild to moderate COVID-19.
574 EFFICACY OF 3TC+DTG VS 3-DRUG REGIMENS IN VIROLOGICALLY-
SUPPRESSED PLWH
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Background: In people living with HIV (PLWH) lamivudine (3TC)+dolutegravir (DTG) maintenance dual therapy (DT) could be less effective than three-drug therapies (TT) with 2NRTIs+DTG in the context of both resistance-associated mutations (RAMs) and lower time of virological suppression (VS). However, data have shown that PLWH who switch to DT usually have longer time of VS than PLWH switching to TT, leading to an immortal-time bias when analyzing observational data. To overcome this issue, we emulated a trial-study design by querying the Italian “ARCA” database.

Methods: PLWH on a TT with PI or a NNRTI, switching to a DTG-based TT or to DT with 3TC+DTG were followed from the first HIV-RNA<50 cp/mL (baseline, BL) up to virological failure (VF) (i.e., >2 consecutive HIV-RNA>50 cp/mL or one HIV-RNA>200 cp/mL). After choosing a grace period of 3 years (i.e., the maximum time of VS after which we supposed an advantage in terms of virologic success for PLWH on DT, based on literature data) we assigned PLWH switching to DT within 3 years to the “treatment”-arm, and PLWH switching to 2NRTIs+DTG (or to DT after 3 years) to the “control”-arm. By using a cloning technique, each participant was also assigned the opposite strategy and censored at the time of deviation from that strategy. By estimating the inverse-probability of censoring weight (IPCW) for each participant, we accounted for the informative censoring triggered by cloning. A Cox regression model was then performed to obtain an unbiased estimate of the effect of DT over TT on VF.

Results: A cohort of 626 PLWH (204 on DT, 422 on TT; 73% men, mean age 44 years) was eligible for analysis (Table 1 summarizes characteristics of study population). The mean time of VS was 5 and 4 years before switch to DT and DTG-based TT, respectively. Overall, 41 VF (10 with DT, 31 with TT) occurred after a mean time of 2.2 and 1.6 years with DT and TT, respectively. A higher crude risk of VF was shown for TT (7.6% versus 4.5% at 2 years; p=0.055). Conversely, the inverse-probability weighted-Cox model indicated a similar risk of VF between DT and TT when no RAMs were present (DT versus TT aHR: 0.88, 95% CI 0.45-1.72; p=0.713) but a higher risk of VF for DT in the presence of M184V/I (versus DT and TT when no RAMs were present (DT versus TT aHR: 0.88, 95% CI 0.45-1.72; p=0.713) but a higher risk of VF for DT in the presence of M184V/I (versus

Conclusion: Previous detection of M184V/I could affect the efficacy of 3TC+DTG as a maintenance strategy.

Table 1. Characteristics of study population at baseline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total study arm (n=626)</th>
<th>3TC + DTG arm (n=204)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, IQR)</td>
<td>43 (IQR 36-50)</td>
<td>44 (IQR 38-50)</td>
<td>0.257</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>297 (48.1)</td>
<td>126 (61.9)</td>
<td>0.033</td>
</tr>
<tr>
<td>Rate factor for HIV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HIV-negative</td>
<td>174 (42.9)</td>
<td>98 (47.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- HIV+</td>
<td>152 (57.1)</td>
<td>106 (52.4)</td>
<td></td>
</tr>
<tr>
<td>- unknown</td>
<td>88 (54.1)</td>
<td>32 (20.5)</td>
<td></td>
</tr>
<tr>
<td>Cumulative efficacy, n (%)</td>
<td>331 (78.8)</td>
<td>158 (73.7)</td>
<td>0.065</td>
</tr>
<tr>
<td>Years since HIV diagnosis, median (IQR)</td>
<td>5 (IQR 3-8)</td>
<td>3 (IQR 3-9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Years of antiretroviral exposure, median (IQR)</td>
<td>22 (IQR 13-39)</td>
<td>12 (IQR 10-33)</td>
<td>&gt;0.050</td>
</tr>
<tr>
<td>Death HIV RNA (log_{10}(cp/mL), median (IQR))</td>
<td>4.57 (IQR 4.40-4.70)</td>
<td>4.73 (IQR 4.50-4.80)</td>
<td>0.136</td>
</tr>
<tr>
<td>Mutation (CD4 count (mmol/L), median (IQR))</td>
<td>217 (IQR 190-284)</td>
<td>217 (IQR 190-295)</td>
<td>0.005</td>
</tr>
<tr>
<td>Previous virological failure (at least one), n (%)</td>
<td>47 (22.9)</td>
<td>22 (10.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CDC stage C, n (%)</td>
<td>53 (26.2)</td>
<td>14 (7.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>ART-naive patients, n (%)</td>
<td>96 (57.0)</td>
<td>21 (10.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RAMs for study drugs at historical genotypes (at least one), n (%)</td>
<td>87 (20.7)</td>
<td>18 (9.6)</td>
<td>&gt;0.050</td>
</tr>
<tr>
<td>l- viral load (cp/mL)</td>
<td>346 (30.0)</td>
<td>154 (27.6)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

576 DRUG RESISTANCE IN PEOPLE FAILING DOLUTEGRAVIR-BASED ART: HIV COHORT COLLABORATION
Tom Loosli1, Stefanie Hossmann2, Suzanne M. Ingle1, Hajra Okhai1, Arv van Sighem3, Melanie Stecher4, Antoinella D’Arminio Monforte5, M. John Gill4, Caroline Sabin1, Gary Maartens9, Huldrych F. F. Günthard10, Jonathan Ac Sterne3, Richard Lessells11, Matthias Egger1, Roger D. Kouyoumdjian12
1University of Zurich, Wettinsgton, Switzerland, 2University of Bern, Bern, Switzerland, 3University of Bristol, Bristol, United Kingdom, 4University College London, London, United Kingdom, 5Stichting HIV Monitoring, Amsterdam, Netherlands, 6University of Cologne, Cologne, Germany, 7University of Milan, Milan, Italy, 8University of Calgary, Alberta, BC, Canada, 9University of Cape Town, Cape Town, South Africa, 10University Hospital Zurich, Zurich, Switzerland, 11Kwazulu-Natal Research Innovation and Sequencing Platform, Durban, South Africa, 12University of Zurich, Zurich, Switzerland

Background: In 2019, the World Health Organization (WHO) recommended dolutegravir (DTG) as the preferred drug for first- and second-line antiretroviral therapy (ART) among all people with HIV (PWHA), including pregnant women and those of childbearing age. DTG has a high genetic barrier to resistance, but PWHA with resistance to nucleoside reverse-transcriptase inhibitors (NRTIs)
who are on (functional) DTG dual- or mono-therapy, and those experiencing challenges adhering to ART, may be at particular risk of DTG resistance.

**Methods:** We pooled data from six European (ATHENA, The Netherlands; Aquitaine cohort, France; Cologne Cohort, Germany; ICONA, Italy; SHCS, Switzerland; UK CHIC, UK), one North American (S Alberta HIV Clinic, Canada) and one South African (AIDS for Aids) cohort to identify PWH who underwent genotypic resistance testing (GRT) while on DTG-based ART. Drug resistance levels and drug resistance mutations (DRMs) were identified using the Stanford algorithm. We assessed associations with DTG resistance using uni- and multivariable ordinal logistic regression, including covariables age, sex, HIV subtype, ART regimen, time on DTG, exposure to integrase strand transfer inhibitors (INSTIs), area under the viral load curve (AUC) and testing frequency, and resistance to RTIs.

**Results:** Among 728 eligible PWH most were from European cohorts (628, 86.3%), men (534, 69.8%) and had HIV subtype B (456, 59.6%). Median time on DTG-based ART was 1.7 years (IQR 0.7 – 3.2); 284 (39.0%) had resistance to RTIs. Ninety-eight (13.5%) had INSTI DRMs with: 8 potential low; 6 low; 19 intermediate; and 6 high resistance levels. DTG monotherapy and NRTI resistance were strongly associated with DTG resistance (Figure). There was some evidence that non-B subtype might be associated with DTG resistance.

**Conclusion:** DTG resistance is rare in PWH failing on a DTG-based ART regimen. It might become a problem with the global scale-up of DTG, particularly in low- and middle-income countries where pre-existing drug resistance is more common, and where individuals remain on failing regimens and are switched to DTG without viral load or resistance testing. Global surveillance of DTG resistance is essential. The mutational pathways require further investigation. Uni- and multivariable ordinal logistic regression models for genotypic DTG resistance levels in PLHIV on DTG-based ART. PLHIV with more than one year of clinical data available prior to the GRT were included (N = 653).

**Complete Results:** A total of 428 participants were selected for sequencing from 1,881 enrolled women. We successfully sequenced 340 participants, of which 193 were from cohort A, 74 from cohort B and 73 from cohort C. At entry, 28.3% of participants had the K103N DRM, 8.8% had K65R, and 11.8% had M184V; cohorts B & C had significantly more DRMs than cohort A. PI DRMs were rarely seen except for M46I/L at low frequency. Cohort B & C participants were more likely to have TF than those in cohort A (Fig. 1A), at TF rates of 26% (A), 41% (B), and 44% (C) (p< 0.001). Presence of K103N at entry significantly increased TF risk among participants on an EFV-based regimen at both high & low frequency, with HR of 3.26 [1.52-6.98, CI 95%] and 2.53 [1.00-6.37, CI 95%] respectively in multivariable analysis stratified by entry viral load (Fig. 1B). For other DRMs at low frequency we found no significant association with TF. At TF, we observed the selection of DRMs in 45% of participants, while remaining participants lacked selection pressure from ART. Only one participant who received a PI-based regimen failed with new PI DRMs. Overall, DRMs to INSTIs were rare, but 1.5% of participants had Q148R at entry despite never taking INSTI drugs.

**Conclusion:** Previous ART experience was associated with increased TF rates, as was K103N at both high & low frequencies for people on NNRTIs. High background of NNRTI DRMs provides additional evidence supporting the transition to INSTI based regimens adopted by Malawi. DRMs and previous ART experience impact treatment failure in pregnant women.

**Complete Results:** A total of 137 participants samples (92 children and 45 adults) with VL ≥ 1000 copies/ml, underwent HIVDR genotyping. The overall prevalence of HIV drug resistance mutations was 71.5% where 78.3% of children and 57.8% of adults had drug resistance mutations. Notably, 5.8% of participants had INSTI drug resistance mutations including major drug resistance mutations; Q148K, E138K, K103N, G140A, T66A, and R263K. Non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside/nucleotide reverse transcriptase inhibitors (NRTI),

**Emerging Integrase Inhibitor HIV Drug Resistance Mutations:**

**Tanzanian National Survey**


1Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; Joint Research Center for Human Retrovirus Infection, Kumamoto, Japan; 2National AIDS Control Program, Dodoma, Tanzania; 3Muhimbili National Hospital, Dar es Salaam, Tanzania; Centers for Disease Control and Prevention, Dar es Salaam, Tanzania; 4Kumamoto University, Kumamoto, Japan

**Background:** In 2019, a potent integrase strand transfer inhibitor (INSTI), dolutegravir (DTG) was made available for public use in Tanzania. However, the switching was conducted without confirmation of the virological suppression, hence the treatment success has not been fully appreciated. HIV drug resistance (HIVDR) including against DTG could be implicated in the notable suboptimal viral load suppression among people living with HIV (PLHIV) in Tanzania. Therefore, we aimed to determine the prevalence and patterns of acquired drug resistance mutations among children and adult populations on antiretroviral therapy (ART) in Tanzania.

**Methods:** We conducted a national cross-sectional HIVDR survey among PLHIV, 866 children (<15 years) on ART for 12(±3) months and ≥ 36 months; and 1173 adults (≥15 years) on ART for 12(±3) months and ≥ 48 months. HIV viral load was estimated using Cobas® 8800 System (Roche Molecular System, Inc. South Branchburg, New Jersey USA). Genotyping was done on DBS and/or plasma of participants with high HIV viremia (VL ≥ 1000 copies/ml). HIV genes (reverse transcriptase, protease, and integrase) were amplified by Polymerase Chain Reaction (PCR) and directly sequenced using Applied Biosystems 3730XL DNA Analyser. The Stanford HIVDR database was used for HIVDR assignment and prediction of phenotypic susceptibility to ART drugs.

**Results:** A total of 137 participants samples (92 children and 45 adults) with VL ≥ 1000 copies/ml underwent HIVDR genotyping. The overall prevalence of HIV drug resistance mutations was 71.5% where 78.3% of children and 57.8% of adults had drug resistance mutations. Notably, 5.8% of participants had INSTI drug resistance mutations including major drug resistance mutations; Q148K, E138K, K103N, G140A, T66A, and R263K. Non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside/nucleotide reverse transcriptase inhibitors (NRTI),
and protease inhibitor (PI) drug resistance mutations were also detected in 62.8%, 44.5%, and 8% of the participants, respectively. In addition, we observed that all the participants with major INSTI drug resistance mutations harbored drug resistance mutations targeting NRTI backbone drugs used in our setting.

**Conclusion:** Taken together, the findings from this survey have revealed that more than 7 out of 10 patients with high HIV viremia in Tanzania have drug resistance mutations. The early emergence of DTG resistance is of concern to the efficacy of the Tanzanian ART program and other similar settings in Sub-Saharan Africa.

579 INCIDENCE OF ACQUIRED INTEGRASE RESISTANCE AFTER TRANSITION TO DOLUTEGRAVIR IN UGANDA


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**Background:** Millions of people with HIV in sub-Saharan Africa have been switched from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) to tenofovir-lamivudine-dolutegravir (TLD) since 2018. Whereas clinical trials have rarely documented resistance to TLD after switch, limited programmatic data are available to estimate the incidence of acquired drug resistance on TLD in the region.

**Methods:** We conducted a longitudinal cohort study of adults with HIV age >18 years in Uganda who were programatically switched from NNRTI-based ART to TLD. We measured plasma HIV-1 RNA viral load (VL) using the Cepheid GeneXpert platform on the day of switch to TLD and at 24- and 48-weeks post-switch. We conducted Sanger sequencing of reverse transcriptase and integrase regions of the pol gene on any plasma specimen with a VL >500 copies/mL.

**Results:** We enrolled 500 participants with a median age of 47 years (IQR 40–53); 41% were women. Sequencing was performed on specimens from 19 participants (Table) with a detectable VL at any of the three study visits. Sequenced participants had a median age of 40 years (IQR 32–52), and 16% were women. At the time of switch to TLD, five participants (1%) had a VL >500 copies/mL, and four of these were sequenced. None had integrase inhibitor mutations prior to switch to TLD. All had NNRTI mutations and three had nucleoside reverse transcriptase inhibitor mutations; yet all were suppressed by 24 or 48 weeks. In follow-up, 1% (n=5/448) and 2% (n=9/483) of participants who completed study visits at 24 and 48 weeks after switch to TLD, respectively, had a VL >500 copies/mL. Although two participants failed with M184V and five participants failed with NNRTI mutations, we did not observe K65R or integrase inhibitor mutations in any individuals after TLD transition. The one individual with M184V alone at 24 weeks resuppressed by 48 weeks. Thus, incidence of acquired integrase resistance in this cohort was 0% (95% CI 0–0.008%) by 48 weeks.

**Conclusion:** No treatment-emergent resistance to dolutegravir was observed after 48 weeks in this large cohort study of ART-experienced adults transitioning to TLD in the Ugandan public sector. Furthermore, two individuals achieved viral suppression despite high-level resistance to both tenofovir and lamivudine at the time of switch from NNRTI-based therapy. These data are reassuring and affirm World Health Organization guidelines for the use of TLD as the preferred ART regimen in resource-limited settings.

580 SUSCEPTIBILITY SCREENING TO bNAbs GS-5423 AND GS-2872 IN ART-SUPPRESSED PARTICIPANTS

**Lisa Selzer,** Laurie A. Vanderveen,* Ayappa Parvanganad,* Ross Martin,* Sean E. Collins,* Megha L. Mehrutra, Christian Callebaut*

*1Gilead Sciences, Inc, Foster City, CA, USA, 2Gilead Sciences, Inc, San Francisco, CA, USA

**Background:** Broadly neutralizing antibodies (bNAbs) display strong antiviral activity by targeting HIV Envelope (Env) with high potency and breadth. However, Env diversity can lead to natural resistance, creating challenges for the use of bNAbs as antiviral therapies, and posing the need to screen participants for susceptibility to bNAbs. We compared genotypic and phenotypic analyses to determine participant susceptibility to GS-5423 (3BN1C17-1S) and GS-2872 (10-1074-L5) prior to enrollment into a Phase 1b study evaluating their safety, tolerability, and efficacy in combination with the HIV capsid inhibitor lenacapavir dosed every 6 months in ART-suppressed people with HIV (PWH).

**Methods:** PBMCs from 124 participants obtained at screening were used to assess susceptibility to GS-5423 and GS-2872 using 3 different methods. Phenotypic analysis of proviral DNA from PBMCs was performed using the PhenoSense mAb DNA assay (Monogram), with susceptibility defined as IC50 ≤ 2 μg/mL. Viral outgrowth in combination with the PhenoSense mAb RNA assay (Monogram) was performed on available PBMCs from 92 participants. The HIV Env gene from proviral DNA in PBMCs was genotyped using deep sequencing (Seq-IT) and susceptibility to bNAbs predicted using previously described Env amino acid signatures (Merculet, et al. 2021).

**Results:** PhenoSense mAb DNA assay results were obtained for 109 of 124 participants (15 assay failures). 75% of participants were susceptible to GS-5423, 65% to GS-2872, and 50% to both bNAbs. Viral outgrowth >1,000 copies/mL was observed for 48 of 92 samples. Phenotypic susceptibility was obtained for 35 of those samples, with 49% susceptible to GS-5423, 69% to GS-2872, and 31% to both bNAbs. Phenotypic susceptibility determinations for outgrowth virus and proviruses were correlated (Fig. 1) (GS-5423 r=0.79, P<0.0001; GS-2872 r=0.75, P<0.0001). Genotypic susceptibility to GS-5423 and GS-2872 was determined for 59 of 109 proviral sequences with phenotypic data. Proportional genotypic signatures predicted phenotypic susceptibility of proviruses and outgrowth virus with high specificity (93-100% GS-5423, 71-96% GS-2872), but low sensitivity (24-11% GS-5423, 76-8% GS-2872).

**Conclusion:** We compared 3 different methods to determine susceptibility to GS-5423 and GS-2872 in ART-suppressed participants. These data demonstrate that the susceptibility to bNAbs is correlated between all 3 assay types and may be useful in further refining the criteria for selecting PWH who could be eligible for bNAbs studies.

Fig. 1: Correlation of neutralization IC50 values between outgrowth viruses and proviruses

581 PHENOTYPIC SUSCEPTIBILITY TO VRC07-523LS AND ITS CORRELATES IN THE ACTG A5357 STUDY


**AIDS Clinical Trials Group**

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**Background:** VRC07-523LS is a broadly neutralizing anti-HIV-1 envelope monoclonal antibody under investigation in combination with LA-cabotegravir for maintenance of HIV-1 suppression in the AIDS Clinical Trials Group (ACTG)
582 HIGH PREDICTED bNAB RESISTANCE AMONG ADULTS WITH HIV-1 SEROCONVERSION IN BOTSWANA

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Background: We used proviral HIV-1 sequences from adults with documented HIV-1 seroconversion in Botswana, to determine HIV drug resistance mutations and predict (in silico) resistance to 33 known broadly neutralizing antibodies (bNAb).

Methods: We analyzed proviral sequences from adults with documented HIV-1 seroconversion (N=140) from a population-based household study (Botswana Combination Prevention Project, 2013-2018). HIV-1C near-full length proviral sequences were generated and adjusted for hypermutations using Hypermut. Surveillance drug resistance mutations (SDRMs) associated with protease inhibitors (PI), integrase strand transfer inhibitors (INSTI), nucleoside reverse transcriptase inhibitors (NNRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) were analyzed according to Stanford Calculated Population Resistance program. HIV gp120 region was used to predict sensitivity of contact sites to 33 known bNAbs using the bNAb-Rep algorithm (https://github.com/RedaRawi/bNAb-ReP). A cutoff of 0.5 probability value was used to classify sensitivity (>0.5) or resistance (<0.5). All gp120 alignments were also used to determine the number of potential N-linked glycosylation sites (PNGS) and compared among bNAb resistant and sensitive strains.

Results: One-hundred-and six (76%) adults with documented seroconversion were ART-naive at the time of sample collection, with a median viral load of 3.9 (Q1: 3.2, Q3: 4.4) log10 copies/mL. Median age was 27 years (Q1: 22, Q3: 33) and most (79%) were female. The overall prevalence of any SDRMs at baseline was 6.6%. We found PI-, NNRTI-, INSTI- and INSTI-associated SDRMs in 1.9% (95%CI 0.2–6.6), 3.8% (95%CI 1.0–9.4), 1.9% (95%CI 0.2–6.6), and 1.9% (95%CI 0.2–6.6), respectively. Prevalence of predicted resistance to 1 or more bNAbs was high among seroconverters (Fig 1); 100% of sequences showed resistance to 2F5, PG16, PGT151 and VRC34.01. In contrast, most sequences (>60%) were likely susceptible to 10-1074, PGDM1400, PCT128, VRC13 and VRC25.25. No difference was observed in the frequency of PNGS compared to bNAb resistance/sensitivity.

Conclusion: We report low levels of transmitted HIV drug resistance mutations but high prevalence of predicted bNAb resistance in adults with HIV-1 seroconversion in Botswana.

Fig 1. Predicted bNAb resistance based on in silico models using HIV proviral sequences from seroconverters in Botswana.

583 STATEWIDE TRENDS OF ACQUIRED HIV-1 DRUG RESISTANCE IN RHODE ISLAND: 2004-2021

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Background: HIV-1 acquired drug resistance (ADR) may compromise sustainable antiretroviral therapy (ART) effectiveness and efforts toward ending the HIV epidemic. However, comprehensive and longitudinal data on ADR, and its current extent and impact on ART options and newer medications is limited and can inform care.

Methods: We aggregated all available HIV-1 protease-reverse-transcriptase-integrase sequences from ART-experienced persons in care in Rhode Island (RI), reviewed their detailed ART histories, and evaluated statewide ADR extent, trends and impact. Drug resistance was evaluated with Stanford Database tools, trends were measured with Mann-Kendall statistic, and multivariable regression analyses were used to evaluate sociodemographic, clinical and ART predictors of impactful resistance.

Results: A total of 1,035 (44% of those with HIV in RI) ART experienced persons (34% female, 42% non-white, 64% men who have sex with men (MSM); mean 6.8 years on ART, exposed to 5.8 drugs and 3.4 regimens; CD4 355 cells/μL) had available sequences in 2004-2021, at least 30 days after ART initiation. Statewide ADR to any drug decreased from 72% to 49% during 2004-2021 (-0.59 Mann-Kendall statistic), with trends mostly driven by NNRTI- (52% to 39%) and NRTI- (58% to 23%), and less by PI- (25% to 6%) associated mutations during those years; with INSTI-associated mutations decreasing from 13% in 2017 to 10% in 2021, all corresponding to ART used in those years. Multiclass
(≥ 2, ≥ 3, ≥ 4) resistance changed from 50%, 12% and 0% in 2004 to 15%, 4% and 1% in 2021 respectively. In 2021, 57.8% of individuals had a three-drug one-pill-once-a-day (OPOD) option, and 93% had a 2-drug OPOD option, with no obvious trends over time for both. People with HIV-1 subtype B were more likely, while MSM and people with longer time on ART were less likely to have ≥2 multiclass resistance. People exposed to a larger number of antiretrovirals and those with HIV-1 subtype B were less likely to have 3-drug OPOD options.

Conclusion: In a unique statewide analysis of longitudinal ADR trends within a densely-sampled HIV epidemic over 2004-2021, we found extensive but decreasing ADR. Lower observed ADR to high-resistance-barrier medications and sustained rates of OPOD eligibility are reassuring; however, continued ADR monitoring is important to maintain ART success, particularly with rising INSTI use in all lines of therapy and 2-drug regimen options.

584 FOSTEMSAVIR RESISTANCE-ASSOCIATED MUTATIONS IN HIV-1C STRAINS FROM BOTSWANA


Background: There is limited data on the prevalence of fostemsavir (FTI)-associated drug resistance mutations in people with HIV (PWH) in Botswana. Botswana is currently experiencing increased cases of patients with MDR HIV strains, which may limit future antiretroviral therapy (ART) options. We here use a large set of HIV-1 C sequences from across Botswana to determine the possibility of the use of FTI-containing regimens by exploring the prevalence of FTI-associated resistance mutations among ART-naive and -experienced individuals in Botswana.

Methods: Previously reported FTI-associated drug resistance mutations (DRMs) were surveyed from 6,030 HIV-1 near full-length sequences generated from participants of the Botswana Combination Prevention Project (BCPP) (2013-2018). Both antiretroviral (ART) naïve and experienced were included. ART experienced individuals were further classified into suppressed (UL<400 copies/mL) and virologic failure (VF) (VL >400 copies/mL).

Results: Among 6,030 HIV-1 gp120 sequences, 1,282 (21.3%) were ART naïve participants while 4748 (78.7%) were on ART at study enrolment. VL data was available for 4,739 (99.8%) among ART experienced, of whom 4526 (95.5%) were suppressed and 213 (4.5%) had VL >400 copies/mL (VF). The overall prevalence of FTR resistance was 13.3% (CI 11.6-15.1). Stratifying the prevalence by ART status, 13.6% (29/213) was reported among ART experienced with VF and 13.3% (170/1282) in ART naïve individuals (p-value=0.9). The most predominant mutations were M434I and M437S reported among 60.3% and 36.2% individuals, respectively. Mutation M434V was higher at ART experienced (10.3%) compared to 1.2% among ART-naïve individuals (p >0.01).

Conclusion: The overall prevalence of DRM DRM was similar in ART naïve and ART experienced individuals in a setting with no prior FTR exposure. We recommend periodic surveillance of FTI-associated drug resistance mutations in order to guide its clinical use in people living with HIV.

585 NO ANTAGONISM OR CROSS-RESISTANCE OBSERVED BETWEEN ISLATEAVIR AND LENACAPAVIR

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Background: Ilatravir (ISL) is a deoxyadenosine analog that inhibits HIV-1 reverse transcription by multiple mechanisms, including inhibition of translocation and delayed chain termination. Lenacapavir (LEN) is a novel capsid (CA) inhibitor that inhibits HIV at multiple points in the viral cycle. Here several translocation and delayed chain termination. Lenacapavir (LEN) is a novel capsid (CA) inhibitor that inhibits HIV at multiple points in the viral cycle. Here several

Results: ISL with LEN demonstrated additive inhibition of HIV-1 replication, with no evidence of antagonism and no significant synergistic or antagonistic effects on cytotoxicity across the range of concentrations tested. ISL exhibited potent (nM) antiviral activity against known LEN-resistance-associated variants and addition of M184V did not alter the antiviral activity of LEN against LEN resistance-associated variants. The combination of reported LEN resistance-associated mutations and M184V did not confer additional potency reductions to ISL beyond M184V alone. In resistance selection experiments, the ISL/ LEN combination more effectively suppressed viral breakthrough at lower multiples of the compounds’ IC50 values and fewer mutations emerged with the combination compared to either compound on its own. The resistance pathways for ISL and LEN were not altered, and no novel mutations emerged that substantially altered the potency of LEN or ISL.

Conclusion: A lack of antagonism and cross-resistance suggest that ISL and LEN can make an effective 2-drug combination for the treatment of HIV.
LONG-TERM HEPATITIS B OUTCOMES IN ZAMBIA WITH TENOFOVIR-TREATED HBV/HIV COINFECTION

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Background: Where HBV/HIV coinfection is most common – sub-Saharan Africa – long-term outcomes have rarely been documented. We evaluated long-term hepatitis B viral, serological, and liver outcomes of tenofovir-based ART in Zambia.

Methods: Adults (18+ years) who were dually HIV antibody and hepatitis B surface antigen (HBsAg) positive were prospectively enrolled at the start of ART (EFV + TDF + 3TC and DTG + TDF + 3TC after 2019). Labs for hepatitis B e antigen [HBeAg], HBsAg, HBV DNA, liver transaminases and HIV (CD4 count, HIV RNA), and transient elastography, were performed at enrollment and at least yearly thereafter. HBV DNA non-suppression (beyond 2 years on ART) and e and s antigen seroclearance were ascertained. Predictors of DNA non-suppression and HBsAg seroclearance were analyzed with multivariable regression. Normalization of ALT elevation and progression of liver fibrosis and cirrhosis, based on TE, were described. In subgroup analysis, we examined the outcomes of patients who were HBeAg-negative, had DNA < 2,000 IU/ml, and no-minimal fibrosis at start.

Results: Among 291 analyzed (median follow-up of 4.9 years), median age was 33 years, 40.9% were women, and 54.3% reported current alcohol use. At enrollment, median CD4 count was 192 cells/mm³; 41.2% were HBeAg-positive, 46.4% had HBV RNA >2,000 IU/ml, 17.7% had significant fibrosis and 6.2% had cirrhosis. DNA non-suppression occurred in 13.5% and was associated with pre-ART WHO stage 3/4 and high DNA and reduced adherence during ART. ALT normalized in two-thirds of patients; however, ALT elevation was present 20-30% of visits beyond 2 years and associated with alcohol use. HBeAg seroclearance was 26.6% at 2 and 34.2% at 5 years. HBsAg seroclearance was 9.2% at 2 and 16.5% at 5 years. No demographic, HBV, HIV, or liver factor examined was associated with HBsAg seroclearance. Regression of fibrosis (80.4%) and cirrhosis (93.8%) was common, progression to fibrosis was rare (2%); none progressed to cirrhosis. In those with markers of inactive HBV and liver disease, both ALT normalization and HBsAg loss occurred on ART.

Conclusion: In Zambia, Tenofovir-based ART effectively controlled HBV in PLWH with higher than expected HBsAg seroclearance. Even those with minimal apparent need for HBV control experienced desired end points with ART. Behavioral interventions for medication adherence and unhealthy alcohol use may be needed to optimize the long-term outcomes of HBV/HIV coinfection.

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"MICRO-INFECTION" OF HBV CAN OCCUR IN MSM WITH VACCINATION OR TENOFOVIR-BASED PrEP

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Background: HBV vaccination and tenofovir-based PrEP prevent acute HBV hepatitis. However, there is little data on prophylactic effect of these HBV prophylaxes against asymptomatic HBV infection. We investigated acute HBV infection and its serological characteristics according to the HBV prophylaxes among an HIV-negative MSM cohort, at sexual health clinic (SHC) in Tokyo, Japan.

Methods: HIV-negative MSM aged 16 years and older were included in SHC. Participants were examined with HBs antigen (HBsAg)/antibody and HBe antibody (HBeAb), HIV infection, syphilis, Chlamydia trachomatis and Neisseria gonorrhoeae infections every 3 months. Those who were diagnosed with acute HBV infection between January 2018 and March 2022 in SHC were subjects for this study. The definition of acute HBV infection was as follows: 1) for those with positive HBsAg at the enrollment of SHC, clearance of HBsAg within 6 months from the first HBsAg positivity, and 2) for those with HBsAg and HBe Ab negativity at the enrollment of SHC, HBsAg or HBeAb serocconversion during the study period. The cases of acute HBV infection were categorized into A) positive HBsAg, B) negative HBsAg and continuous HBeAb positivity, and C) negative HBsAg and transient HBeAb positivity. B) and C) were analyzed by HBV prophylaxes using prior HIV vaccination and tenofovir-based PrEP with t-test or chi-square test. HBV DNA was measured among C) if available serum samples were stocked.

Results: A total of 1972 MSM were included in SHC as of March 2022. Among them, 48 (mean age 31 years) were diagnosed with acute HBV infection. As shown in the Table, MSM with transient HBeAb positivity were significantly more likely to have HBV prophylaxes, while MSM with symptomatic hepatitis and HBsAg positivity were not observed among those who had had HBV prophylaxes. MSM with transient HBeAb positivity was older than MSM with continuous HBeAb positivity, which may be reflected by the fact that MSM with the HBV prophylaxes were significantly older (39.3 vs 28.3 years, p < 0.001). Of 11 MSM with transient HBeAb positivity, 3 cases were tested for HBV DNA and it was detected in one case. The average time to disappearance of HBsAg was 174 days.

Conclusion: While HBV prophylaxes prevented symptomatic hepatitis, the infection without serological trace occurred. Clinical significance of this “micro-infection” should be investigated in further studies.

Detail of acute HBV infection according to serology and prophylaxes in MSM

BULEVIRIDE +/- PEIFN IN HIV/HBV/HDV COINFECTED PATIENTS IN REAL-LIFE SETTINGS

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1cATU Study Team
2Centre Hospitalier Universitaire de Bordeaux, Pessac, France, 3Assistance Publique–Hôpitaux de Paris, Paris, France, 4Centre Hospitalier Universitaire de Grenoble, Grenoble, France, 5Centre Hospitalier Universitaire de Saint-Brieuc, Saint-Brieuc, France, 6Centre Hospitalier d'Auxonne, Auxonne, France, 7Centre Hospitalier Universitaire de Tours, Tours, France, 8Centre Hospitalier d'Avignon, Avignon, France, 9Centre Hospitalier Intercommunal de Creteil, Creteil, France, 10Centre Hospitalier de Saint Brieuc, Saint Brieuc, France, 11Assistance Publique–Hôpitaux de Paris, Creteil, France

Background: Buleviride (BLV) is a lipopeptide inhibiting the HBV/HDV entry into the hepatocytes. Significant HDV RNA decline was observed in HIV/HBV/HDV patients who received 48 weeks of BLV in monotherapy or in combination withPEG-interferon a 2a (PEG-IFNa). Until now, no data has been presented in HIV/HBV/HDV co-infected patients treated with BLV. The aim of this analysis was to evaluate the efficacy and safety of BLV 2mg daily with or without PEG-IFNa 2a...
for 12 months in HIV co-infected patients treated in the French BLV early access program.

**Methods:** 21 HIV patients (male 71%, mean age 48 years, cirrhosis 52%, median HDV-RNA 6.09 log10 IU/mL, median HIV-RNA 0 cp/mL (three patients had detectable HIV-RNA at day 0, all < 100 cp/mL, median CD4 558/mm3) with chronic HIV/HBV/HDV infection, with compensated cirrhosis/severe fibrosis or moderate fibrosis with elevated ALT levels, were included in the French early access program. Patients received BLV 2mg QB SC alone (N=13) or in combination with PEG-IFNα once weekly (N=8) according to physician’s choice. HIV drugs regimen (DR) were TAF/FTC (in 100% of patients), and either NRTI, PI, INI, or NNRTI.

**Results:** No specific adverse events were reported and no HIV drug modification was needed (median CD4 at M12: 540/mm3). Only one patient had detectable HIV-RNA at M12 (31 cp/mL at baseline and 54 cp/mL at M12). Early discontinuation (before M12) was observed in 8 (38%) patients: 2 adverse events (variceal bleeding, rectal cancer), 3 lost to follow-up or patient decision, 3 other reasons. From baseline, HDV RNA (log10, IU/mL) declined overtime: M3 -2.25, M6 -4.09, M9 -3.37, and M12 -4.19. Main results at M12 are indicated in Table.

**Conclusion:** In this first real-world cohort of HIV/HBV/HDV patients, daily administration of BLV 2 mg for 12 months was safe and well tolerated with no impact on CD4 and HIV viral suppression. Strong HDV antiviral and biochemical responses were observed in real-life irrespective of the BLV regimen administered.

**Virological and Biochemical response at Month12**

<table>
<thead>
<tr>
<th>Results at M12</th>
<th>BLV monotherapy</th>
<th>BLV + PEG-IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ALT level (&lt; 40 IU/L)</td>
<td>5/8 (62.5%)</td>
<td>3/7 (42.9%)</td>
</tr>
<tr>
<td>2 log decrease from baseline or undetectable HDV-RNA (%)</td>
<td>7/7 (100%)</td>
<td>5/7 (71.4%)</td>
</tr>
<tr>
<td>Undetectable HDV-RNA (%)</td>
<td>6/7 (85.7%)</td>
<td>5/7 (71.4%)</td>
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**590 HCV INCIDENCE AMONG MSM ON PrEP AND MSM WITH HIV IN NEW YORK CITY OVER 2 DECADES**

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**Background:** Despite HCV elimination efforts, transmission among MSM with HIV has continued at a high rate in most locales. Recently, MSM on PrEP have emerged as a risk group, significantly expanding those at risk for sexual acquisition of HCV. New York City (NYC) has been an epicenter of this HCV epidemic, but there are no data quantifying the extent of this epidemic in the US.

**Methods:** We performed a retrospective analysis of the electronic health records from across the Mount Sinai Health System for incident HCV infections among MSM (2000-2022). The primary comparison was MSM with HIV and MSM on PrEP. Incident HCV infection was defined as a positive HCV Antibody test after an initial negative Ab test. Incidence rates were calculated using the time between the initial negative Ab test and either a positive Ab test or the last negative Ab test. Kaplan-Meier (KM) analysis was used to compare cumulative probability of incident HCV infection.

**Results:** Records were available for 20,844 MSM. Among those with a baseline HCV Ab test and at least one follow-up Ab test >30 days later, 6443 were MSM with HIV, 1728 were MSM on PrEP, and 503 were MSM not on PrEP. Among MSM with HIV, there were 271 incident HCV infections over 32,693 years of follow-up [mean 5.1 (SD 3.9) years]. Among MSM on PrEP there were 43 incident HCV infections over 4540 years of follow-up [mean 2.6 (SD 1.9) years]. There was no difference between the HCV incidence rates for these groups (0.83 (95% CI 0.73, 0.93)/100 PY; 0.95 (95% CI 0.69, 1.28)/100 PY, respectively, p=0.32). Similarly, KM analysis showed no difference in cumulative probability of HCV infection between MSM with HIV (blue) and MSM on PrEP (red) (p=0.08) (Figure). Time trend analysis suggested no declines in incidence rates during the study period for either group. There were just three incident HCV infections among MSM not on PrEP [incidence rate 0.12 (95% CI 0.03, 0.35)/100 PY], significantly lower than for MSM on PrEP.

**Conclusion:** Less than a decade after the FDA approval of PrEP, and despite the availability of curative all-oral HCV treatment, touted as the tool sufficient to eliminate HCV among MSM, the incidence rate of HCV infection among MSM on PrEP in NYC is equivalent to that of MSM with HIV. Our data indicate that active HCV surveillance is needed for MSM on PrEP, and the CDC PrEP guidelines, which do not adequately address HCV testing, should be updated. Most importantly, further HCV risk reduction and prevention efforts are needed for both MSM on PrEP and MSM with HIV.

Kaplan-Meier Curve of Incident HCV Infections among MSM on PrEP and MSM with HIV in a NYC Health System

**591 HEPATITIS C BURDEN IN GREECE, ITALY, PORTUGAL, AND SPAIN: 2000-2019**

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**Background:** Viral Hepatitis remains a health priority. We performed a comprehensive evaluation of epidemiological HCV estimates in Southern countries of Western Europe and assessed the impact of the 2008 economic crisis on HCV burden.

**Methods:** We analyzed data of the Global Burden of Diseases to describe the patterns of six measures of HCV burden [prevalence, incidence, mortality, years lived with disability (YLDs), years of life lost (YLLs), disability adjusted life years (DALYs)] in Greece, Italy, Portugal, Spain. We assessed age-standardized rates (per 100,000 population) between 2000-2019, disaggregated by sex and age, and compared the annualized age-standardized rate of change (ARC%) in 2000-2010 (pre-austerity) and 2010-2019 (post-austerity).

**Results:** Prevalence, incidence and YLDs rates of acute HCV showed a general stable trend in Western Europe (WE), globally and in the four studied countries except Italy, where, despite a marked decline (ARC 1.4% in 2010-2019), the 2019 estimates [7.8 (95% UI 6.6-9.2)] were still 1.7-fold higher than in WE. Mortality, YLLs and DALYs associated with acute HCV decreased in the analyzed countries and peaked in Greece post-austerity. Globally and in Greece, mortality rate was higher in females than in males (1.3-times and 1.5-times in 2019, respectively). Mortality caused by chronic liver diseases including cirrhosis decreased globally, in WE and in all countries albeit at a lower rate in the post-austerity period (decrease in ARC for WE 2.5% in 2000-2010; 1.6 in 2010-2019).

Lever cancer prevalence due to HCV increased in WE (ARC 2.1%) and in the analyzed countries mainly in the pre-austerity period except for Italy. However, despite having the highest prevalence rate in both sexes, Italy showed major decreases in all six-disease metrics. HCV liver cancer mortality declined significantly only in Italy (ARC 2.6%) and globally (ARC 2.1%) especially in the pre-austerity period, while Portugal experienced a major increase post-austerity. Overall, males and people over 70 years old are at greater risk of developing chronic liver diseases due to HCV infection.

**Conclusion:** The economic crisis of 2008 negatively impacted hepatitis C related liver cancer mortality rates in Greece, Italy, Portugal and Spain. Despite the observed recovery in recent years, elimination of HCV infection by 2030 will be a major challenge in these countries and the COVID-19 pandemic and the current grim economic context are expected to compromise even further hepatitis C elimination.
592 USABILITY AND ACCEPTABILITY OF HCV SELF-TESTING IN THE GENERAL POPULATION FROM BRAZIL

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Background: Hepatitis C virus self-testing (HCVST) has been recommended by the World Health Organization (WHO). However, data on the feasibility of HCVST in Latin America remain scarce. Therefore, we aimed to assess the usability and acceptability of HCVST among the general population in the Primary Health Care (PHC) in Rio de Janeiro (Brazil).

Methods: This cross-sectional study was conducted in a Basic Health Unit in Rio de Janeiro from 04-Jul-2022 to 13-Sep-2022. Participants had access to written instructions for use and a video with a step-by-step oral fluid HCVST kit (OraQuick® HCV Self-Test). A trained healthcare worker (HCW) observed the participant performing the test. After HCVST, the HCW performed a second HCV testing using the same kit. Usability was assessed by observing errors made and difficulties faced by participants. A post-testing questionnaire assessed the acceptability of HCV self-testing. Inter-reader (re-reading) and inter-operator (re-testing) concordance were assessed.

Results: A total of 553 participants (75% female; median age = 52 (IQR: 39-61) years, 51.7% with schooling < 10 years; 16.8% with diabetes) were included. People correctly opened the kit package, inserted the tube in the tube support, and collected oral-fluid samples in 95.1%, 90.2%, and 69.3%, respectively. After sample collection, correct insertion of the flat pad in the tube and correct timekeeping for result interpretation were observed in 86.8% and 93.5% of cases, respectively. A total of 62.2% (n=344) of participants completed the HCVST process without assistance from an HCW. Inter-reader agreement of HCVST results was 94.4%, with a Cohen’s kappa of 0.52 (n=550) (Table). The agreement between participant-reported HCVST results and HCW-administered oral fluid HCV rapid test results was 99.6%, with a Cohen’s kappa of 0.67 (excluding invalid tests, n=500) (Table). A total of 91.5% felt safe performing HCVST. Most participants rated the HCVST process as easy (74.1%) or very easy (23.3%), 98.7% reported that they would be willing to use HCVST again, and 99.6% would recommend HCVST to their family, sexual partners, or friends. A total of 70.7% would prefer to perform HCVST in a health unit, and few people would like remote/digital (14.8%) or presential (28.6%) assistance by an HCW for HCVST.

Conclusion: We demonstrated high usability and acceptability of oral fluid HCVST in a large sample of the population in a PHC in Rio de Janeiro. Table: Inter-reader (re-reading) and inter-operator (re-testing) concordance of HCVST stratified by age and schooling (n=553)

593 UPTAKE AND TAKE CARE TO HCV SELF-TESTING: RESULTS FROM A MULTI-COUNTRY RCT

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Background: Globally, of the estimated 58 million people living with the hepatitis C virus (HCV), only 23% know their status. Scale-up of HCV testing is needed to close this diagnostic gap and aid in the achievement of the WHO 2030 elimination goals. There are currently no data on the real-world impact of HCVST. We evaluated the impact on uptake of HCVST compared to standard of care in 3 different HCVST models in Georgia, Malaysia, and Pakistan.

Methods: Multiple modalities of HCVST were evaluated across the project countries. This included: 1) in Georgia: courier delivery of HCVST (for men who have sex with men [MSM]), peer delivery of HCVST (for MSM and people who inject drugs [PWID]), and the standard of care - referral to HCV testing facility; 2) in Malaysia (for those who self-identify as a key population): courier delivery of HCVST, and standard care - referral to HCV testing facility; 3) in Pakistan (general population): home distribution of HCVST or standard care of hospital-based HCV screening. To understand the relative impact in the different modalities of HCVST in HCV diagnosis, we evaluated the HCV care cascade for each subgroup in each modality of care compared to the standard of care.

Results: In all 3 countries across all subpopulations, uptake of HCV Ab testing was higher in the HCVST groups compared to the control group (Table 1). Linkage to care was better in the HCVST arms as compared to the standard of care in Malaysia and Georgia for all sub populations and self-test modalities. In Pakistan, while the linkage to care was technically lower for self-test compared to standard of care, the total number of people successfully diagnosed was more than 10-fold greater. Results on uptake and linkage for HCVST compared to standard care did not differ significantly by sex.

Conclusion: Across all HCVST modalities in all three countries, HCV testing uptake was greater with self-testing modalities as compared to the standard of care. Further to that, linkage to care was high across HCVST modalities. Careful consideration is needed for further scale-up of HCVST including: expected HCV Ab prevalence among target groups (affecting underlying cost-effectiveness of HCVST modalities), provision of tools to ensure testers are able to perform and interpret the test correctly, and mechanisms to facilitate psychosocial support and linkage to care.

Table 1: preliminary results across Georgia, Malaysia, Pakistan

594 MOBILIZING PRIMARY CARE PROVIDERS TO TREAT HEPATITIS C IN RURAL WEST VIRGINIA

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Background: WV has ranked 1st or 2nd for acute HCV incidence for over a decade but has few specialists and severe transportation challenges, contributing to
in equitable access to HCV care. Most clinicians trained before 2016 are unaware that HCV is readily curable.

Methods: We developed the WV Hepatitis Mentoring Partnership (WVHAMP) to empower primary care providers (PCPs) to manage HCV by training and mentoring them through the cascade of care. WVHAMP supports health equity in underserved areas as well as also meeting Medicaid’s initial requirement for treatment under special guidance. PCPs engage with HCV experts via web-based training combined with a customized, HIPAA-compliant, web-based data system for case-based bidirectional communication. Using an informatics platform available to all partners supports shared decision-making, fostering a learning network for HCV care and increasing self-efficacy and efficiency while enabling patients to receive care in the community from clinicians they know and trust.

Results: Since 3/2020, WVHAMP has trained 150 providers and responded to 761 consults. Of the 261 patients who have reached the SVR12 timepoint, 257 (98.5%) have been cured by PCPs who have never previously treated HCV, a rate similar to that of specialists (95-99%).

Conclusion: WVHAMP Scholars, who have achieved an SVR12 rate comparable to experts, are contributing to HCV microelimination in underserved rural communities. This model can be readily expanded to HCV care in underserved urban areas as well.

595 OPTIMAL FREQUENCY OF HCV RNA TESTING FOR PLWH AT RISK FOR ACUTE HCV INFECTION

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Background: Timely diagnosis of acute HCV infection with subsequent treatment with direct-acting antivirals among at-risk populations is the most effective and feasible way to cease HCV transmission. However, the optimal frequency of HCV RNA testing has rarely been assessed among people living with HIV (PLWH).

Methods: In this multi-center study involving 11 hospitals designated for HIV care around Taiwan, we conducted 3-stage pooled plasma HCV RNA testing every 3 months among PLWH who were at high risk for acute HCV viremia, including those recently diagnosed with sexually transmitted diseases (STDs), those with HCV clearance spontaneously or with antiviral therapy, and those with elevated aminotransferases. Repeat enrollment was allowed. We estimated the incidence rates of HCV viremia during the 1-year follow-up and examined the proportions of delayed detection of HCV viremia if the testing frequency had been changed from every 3 months to every 6 or 12 months.

Results: A total of 1,875 PLWH were enrolled between June 2019 and August 2022; 99.9% were men and 98.3% were men who have sex with men (Table). At enrollment, 74.7% of the participants were included because of recent STDs, 25.2% and 3.8% because of HCV viral clearance by antiviral therapy and spontaneously, respectively, and 15.4% because of elevated aminotransferases. A total of 108 cases of HCV viremia were detected during the study period, with 63 cases (58.3%) detected at enrollment and the remaining 45 cases (41.7%) detected during 1,208.11 person-years of follow-up (PYFU), which led to an incidence rate of HCV viremia of 37.25 cases per 100 PYFU. The mean HCV RNA load of the 45 incident cases was 5.41 (range, 1.30-7.77) log10 IU/mL. If the testing frequency had been changed to every 6 or 12 months, respectively.

Conclusion: With HCV RNA testing performed every 6 or 12 months among high-risk PLWH, the detection of a large proportion (62.2-91.1%) of PLWH who were recently HCV-12 outcomes for novel HCV genotype/subtypes with sof/vel in minmon study

Win Min Han, Sunil S. Solomon, Sandra Wagner-Cardosa, Jiani Li, Ayappa Parvangananda, Mark S. Sulkowski, Susanna Naggie, Ross Martin, Hongmei Mo, Evgenia Maiorova, David Wyles

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Background: The characterization of response to direct antiviral agents for rare or novel HCV subtypes is important for global elimination strategies. Genotypes (GT) 1, 2, 3, 4 and 6 are comprised of multiple subtypes and display a high degree of genetic variability. Here, we describe the identification of novel GT-4 subtypes and a GT-7 HCV isolate distinct from 7a and 7b and their sustained virologic response (SVR) outcomes.

Methods: ACTG study A5360 (MINMON) was a phase 4 open-label study of minimal monitoring with 12 weeks of sofosbuvir/velpatasvir for treatment-naive HCV infection. Four hundred participants were enrolled across 38 sites in Brazil (n=131), South Africa (n=12), Thailand (n=110), Uganda (n=15) and the USA (n=152). Amplicon-based deep sequencing analyses of NS3, NS5A and/or NS5B were conducted for all baseline samples. HCV genome sequencing was used random primer Nugeg phase 2 sequencing, sequencing was performed on plasma samples which failed standard amplification. Nucleotide Blast analysis followed by phylogenetic analysis was used to confirm HCV subtype.

Results: In MINMON study, SVR12 was achieved in 96% (379/399) of participants. For most participants enrolled, a specific HCV subtype was assigned based on a close genetic relationship to previously described subtypes. However, for 1 patient from Uganda, sequences obtained from plasma HCV RNA showed <85% sequence homology to all known HCV subtype reference strains. Blast analyses of publicly available datasets revealed close homology to a provisional GT-7c HCV isolate also originating from Uganda (KU861177). In addition, there were N=5 patients from Uganda and South Africa with GT-4 HCV that showed <85% amplicon homology to all known GT-4 subtypes. Presence of NS3, NS5A and NS5B resistance associated polymorphisms in GT-4 with novel subtypes and GT-7 HCV isolates are summarized in Table 1. All participants with novel subtypes of Gt-4 and GT-7 HCV treated with sofosbuvir/velpatasvir achieved SVR12 despite the presence of NS5A RAVs and NS5B nucleos(t)ide RAVs (100% SVR12).

Conclusion: A new strain of HCV subtype 7c and novel subtypes of GT-4 were identified. Sofosbuvir/velpatasvir treatment for 12 weeks was safe and efficacious against these novel strains of HCV. These findings highlight even greater genetic diversity of HCV subtypes than previously recognized. Global HCV elimination strategies need to account for a growing understanding of HCV diversity.
Table 1. NS5, NSSA and NSSB Resistance Associated Polymorphisms in novel genotype 4 subtypes and genotype 7 HCV isolates.

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597 SOFOSBUVIR/VELPATASVIR PHARMACOKINETICS IN PREGNANT WOMEN WITH HEPATITIS C VIRUS

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Background: Treatment of hepatitis C virus (HCV) with direct acting antivirals during pregnancy could cure maternal HCV during antenatal care engagement and prevent perinatal HCV transmission. A study of ledipasvir/sofosbuvir in pregnancy (NCT02683005) showed good efficacy and favorable pharmacokinetics (PK), but a pan-genotypic regimen was needed. Our objectives were to compare the PK parameters of sofosbuvir/velpatasvir (SOF/VEL) in pregnant versus nonpregnant women, and to assess delivery outcomes and virologic response.

Methods: In this open-label, phase 1 study, HCV-negative pregnant women with chronic HCV infection were enrolled between 23-25 weeks’ gestation. At entry, participants began SOF-400mg/VEL-100mg daily for 12 weeks. Twenty-four-hour intensive PK visits were performed at 3 and 9 weeks of treatment. VEL, SOF and GS-331007 (the inactive metabolite of SOF) in plasma were measured using validated UPLC-MS/MS assays. PK parameters were determined using non-compartmental methods (Phoenix v8.3) and geometric mean ratios and 90% CI compared to historical intensive PK data in non-pregnant women from registrational trials. Maternal adverse events, delivery outcomes, and the sustained virologic response 12 weeks after therapy (SVR12) were also determined.

Results: Fourteen participants were screened, 3 were excluded due to declining participation, having a fetal anomaly and spontaneous HCV clearance, and 11 participants enrolled. One participant discontinued treatment after two doses due to worsening of hyperemesis. VEL exposures and SOF maximum concentration (Cmax) were similar to historic data, but SOF area under the curve (AUC) was 38% lower, respectively (Table). Consistent with SOF/ledipasvir in pregnant women, lower GS-331007 exposures across Kinshasa, DR Congo were observed and possibly due to increased glomerular filtration rate during pregnancy. A multicenter study to evaluate SOF/VEL safety and efficacy in pregnant women with chronic HCV infection was underway (NCT05140941).

Conclusion: Sofosbuvir/velpatasvir (SOF/VEL) in pregnant women was well tolerated and demonstrated similar virologic response. These results support the use of SOF/VEL in pregnant women with chronic HCV infection.
**PREVALENCE AND EVALUATION OF HEPATITIS B VIRAL REPLICATION IN PREGNANT WOMEN IN MALI**

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**Background:** Pregnant women with a high Hepatitis B Virus (HBV) DNA viral load (VL) can still transmit HBV to the fetus or newborn, despite receiving hepatitis B immunoglobulin (HBIG) and vaccination of the newborn at birth. Although HBV infection is common in sub-Saharan Africa, data on maternal virological outcomes are limited.

**Methods:** In this longitudinal study, we assessed the sero-prevalence of hepatitis B surface antigen (HBsAg) from pregnant women's samples obtained during antenatal visits between January and May 2022 at a public health clinic in Bamako, Mali. HBsAg positive samples were then tested for HBV VL with a high VL defined as >2000 IU/mL.

**Results:** Of the 998 pregnant women included, 84 (8.4%) had a positive HBsAg. Of these 84, median age was 27 yrs (interquartile range [IQR], 23–32); most were married (98%) and homemakerers (73%); and 18% were primiparous, however, only 10% knew their HBV serological status before the current pregnancy. Alanine aminotransferase (ALT) level was <35 IU/L in 92% of cases and 26 (34.6%) had a VL > 2000 IU/mL, including 5.3% with a VL > 200 000 IU/mL. Three (3.5%) were HBV co-infected of which two had a detectable HIV VL >40 and one had HBV DNA at 1500 IU/mL. There was no statistically significant relationship between age, parity, and VL (p<0.05).

**Conclusion:** This study characterizes HBV infection among pregnant women in Mali. HBsAg seropositivity is high and a third of women had HBV VL >2000 IU/mL where antiviral treatment would be indicated to prevent mother to child transmission of HBV. These data confirm the need for hepatitis B vaccination and treatment programs for women of child-bearing age in sub-Saharan countries like Mali, as well as, routine HBs Ag screening of pregnant women.

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**EPILOGICAL HISTORY OF HEPATITIS B VIRUS SUB-GENOTYPE D3 IN BOTSWANA**


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**Background:** Botswana has an intermediate Hepatitis B Virus (HBV) prevalence of 2-7%. To date, three HBV genotypes (A, D and E) have been reported in Botswana at the prevalence rates 80%, 18.6%, and 1.4%, respectively. However, the evolutionary history of all the identified genotypes within the population in Botswana are not well characterized. Such evolutionary analysis of HBV/D3 within Botswana population helps in tracking disease evolution over time hence aiding in theoretical HBV prevention and management strategies. Our study first describes the origins, introductions (Time to Most Common Recent Ancestor (tMRCA), and evolutionary patterns of HBV sub-genotype D3 circulating in Botswana. Furthermore, we investigate the pairwise diversity on this sub-genotype and its spread within the population.

**Methods:** Analysis was carried out using 69 available, HBV/D3 near-full-length sequences retrieved from the NCBI database of which 24 were from Botswana. Population dynamic analysis of the HBV/D3 (HBV/D3) sequences amongst people with hepatitis B virus (for PWH) was performed using the Bayesian coalescent model as implemented in BEAST2 software. The temporal signal was estimated through the root-to-tip method using node density in tempEst v1.5.3 and the correlation coefficient was used to indicate the amount of variation in genetic distance explained by sampling time. Skyline plots were used to estimate the effective HBV/D3 infections in Botswana population over time. Diversity analysis of Botswana sequences was done based on pairwise distances analysis implemented in MEGA v.7.0.1 software. Botswana sequences were partitioned into 7 HBV open reading frames (ORFs) and used to calculate nucleotide diversity.

**Results:** HBV/D3 tMRCA amongst PWH in Botswana dated back to 1964 (1839–1989), 95% Highest Posterior Density (HPD). Skyline plot showed a sharp increase in the number of HBV/D3 infections around the years 1999 and 2000 which is over the last approximately 22-23 years ago. The Precore region had the highest median diversity of 0.02652 (IQR, 0.0115–0.025) and the surface (S) region was relatively conserved with median diversity of 0.0074 (IQR, 0.004–0.0134) p < 0.01.

**Conclusion:** This study provides new insights of HBV/D3 phylogeographic information in Botswana by predicting its tMRCA, origin and diversity thereby revealing the evolutionary dynamics of the HBV genotype. Diversity analysis showed that core region was the most diverse region and confirmed that the surface region was the most conserved region.

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**HEPATITIS B TREATMENT OUTCOMES AMONG PEOPLE WHO INJECT DRUGS IN KENYA**

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**Background:** Hepatitis B virus (HBV) is a growing public health concern with approximately 1.5 million infections occurring annually globally. Direct-acting antivirals (DAAs) are highly efficacious in treating HBV and strategies tailored for disproportionately affected groups such as people who inject drugs (PWID) are needed for early identification and treatment of infected individuals.

**Methods:** We assessed factors associated with HBV treatment success among PWID receiving ledipasvir-sofosbuvir at needle syringe programs (NSP) and methadone clinics in 3 Kenyan counties. An observational cohort study investigated whether treatment success, measured by sustained viral response 12 weeks after treatment completion (SVR12), differed between daily directly observed therapy (DOT) delivered at NSPs versus methadone clinics. Negative binomial regression was used to compare the proportion of clients who were treated and achieved a SVR12 between NSPs and methadone programs.

**Conclusion:** Treatment success was associated with a lower likelihood of SVR12 compared to traveling ≤20 minutes (aRR=0.65, p=0.004, and aRR=0.66, p=0.027, respectively). Longer travel time to reach the treatment site (≥21-30 minutes and >45 minutes) was associated with a lower likelihood of SVR12 compared to traveling ≤20 minutes (aRR=0.65, p=0.004, and aRR=0.66, p=0.027, respectively).

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**HEPATOCELLULAR CARCINOMA SCREENING AMONG HIV/HBV-COINFECTED INDIVIDUALS IN ZAMBIA**

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**Background:** Chronic hepatitis B virus (HBV) infection is the most common cause of hepatocellular carcinoma (HCC) in sub-Saharan Africa (SSA). Guidelines recommend 6-monthly abdominal ultrasound (AUS) screening for adults of African origin living with chronic HBV, but few screening programs exist in SSA. We evaluated the uptake and outcomes of HCC screening in a cohort of people.
behaviours (Figure). HCV reinfection incidence was highest for those with sustained high IDU (33.0/100 PY; 95%CI 17.7, 61.3) and chemsex behaviours (23.3/100 PY; 95%CI 14.5, 37.5). STI incidence was highest for those with sustained high-sex-group behaviour (161.1/100 PY; 95%CI 136.4, 190.2).

Conclusion: Limited behavioural change was observed following treatment for recent HCV infection. Findings support regular reinfection surveillance and rapid access to retreatment.

Figure. Group based trajectory modelling of sexual behavioural outcomes among gay and bisexual men. Behavioural trajectories for (A) condomless anal intercourse with casual male partners, and (B) group-sex, during, and following treatment for recent HCV infection. The legend includes the proportion of the population assigned to each trajectory. Abbreviations: CAI-CMP, condomless anal intercourse with casual male partner; SCR, screening; BSL, baseline; ETR, end or treatment; SVR12, sustained virological response testing 12-weeks post-treatment; FU, follow-up
LONG-TERM LIVER EVENTS IN PATIENTS WITH HCV CHRONIC INFECTION ACHIEVING SVR

OBJECTIVES: To assess the incidence of liver complications after achieving sustained viral response (SVR) in patients with hepatitis C virus (HCV) chronic infection treated with direct-acting antivirals (DAAs).

MATERIALS AND METHODS: Multicenter cohort study involving 17 hospitals in Spain. HCV-monoinfected and HCV/HIV-coinfected individuals were included. The main inclusion criteria were: 1) Liver stiffness (LS) prior to treatment ≥ 9.5 kPa; 2) Achieved SVR with DAA-based therapy; 3) Available LS measurement at SVR. Patients were considered for analysis if they achieved SVR without failure in the prediction of liver decompensation.

RESULTS: One thousand one hundred and eleven patients were included. Six hundred and eight patients were included, 374 (62%) were living with HIV. Twenty-seven (4.4%) individuals developed liver decompensation (LD) after achieving SVR. Among HIV/HCV coinfected individuals, Baveno VII criterion HR < 14 KPa at the time of SVR achieved NPVs of 100% (96.5%-100%) and 100% (98.4%-100%), avoiding 17% and 38% of LD surveillance measures being avoided. Among HCV monoinfected patients, Baveno VII and LS< 14 KPa criteria maintained NPV at 100%.

CONCLUSION: Liver complications continued to occur after achieving SVR, although the overall incidence is low. HCC remained the most frequent event. Three years after SVR, HCC and PHGB developed more frequently, while cases of ascites and EH emerged as a consequence of other toxic and/or metabolic causes. To identify patients who would benefit from long-term HCC and PHGB screening and thus design cost-effective surveillance programs for liver events is a high priority.

PREDICTION OF HEPATIC DECOMPENSATION AFTER SVR IN PATIENTS WITH HCV CHRONIC INFECTION

OBJECTIVES: To assess the incidence of liver complications after achieving SVR in patients with hepatitis C virus (HCV) chronic infection treated with direct-acting antivirals (DAAs). Patients were considered for analysis if they achieved SVR without failure in the prediction of liver decompensation.

MATERIALS AND METHODS: Multicenter prospective cohort study. Patients in the GEHEP-011 cohort who: 1) pre-treatment HR ≥ 14 KPa; 2) non-LD prior to SVR. We assessed the diagnostic accuracy of LS < 14 KPa and Baveno VII criteria (favorable status HR < 12 KPa and platelet count > 150,000 x 10^6/mL) at the time of SVR for predicting PHT-related LD. Non-predicted LD, negative predictive values (NPV) and the proportion of patients in whom screening for such LD (liver elastography and upper GI endoscopy) would be discontinued were specifically evaluated.

RESULTS: Six hundred and eight patients were included, 239 (62%) were living with HIV. Twenty-seven (4.4%) individuals developed liver decompensation (LD) after achieving SVR. Among HIV/HCV coinfected individuals, Baveno VII criterion HR < 14 KPa at the time of SVR achieved NPVs of 100% (96.3%-100%) and 100% (98.4%-100%), avoiding 17% and 38% of LD surveillance measures, respectively. Tackling into account HIV coinfection, the performance of the two criteria was similar. In HCV monoinfected patients, Baveno VII and RH < 14 KPa criteria maintained NPV at 100%. Among HIV/HCV-coinfected individuals, the NPV of these criteria for coinfection were also 100% (94.0%-100%) and 100% (907.5%-100%) for Baveno VII and RH < 14 KPa, respectively. In both settings, their use resulted in 16% and 39% of LD surveillance measures being avoided.

CONCLUSION: Among HIV/HCV coinfected individuals, Baveno VII criterion also identifies patients at low risk of developing liver complications after achieving SVR. The RH< 14 KPa criterion presents a better diagnostic performance than Baveno VII, as it identifies a larger number of patients unlikely to develop LD. If this criterion were considered, LD surveillance measures could be spared in more than one third of cirrhotic patients with SVR, without failures in the prediction of these events.
607 LONG-TERM CHANGES IN LIVER STIFFNESS POST-SVR IN PATIENTS WITH HCV CHRONIC INFECTION

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GEHEP-011
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Background: In patients with HCV chronic infection, the achievement of sustained viral response (SVR) is related to a marked reduction in liver stiffness (LS), which is associated with a decrease in the incidence of liver events. During HCV active infection, LS correlates with the severity of liver damage and adds prognostic information. After SVR, LS also identifies individuals with no risk of developing liver complications in the short term. However, Information on long-term changes in LS in this subset is scarce.

Methods: Multicenter ambispective cohort study (17 hospitals in Spain). HCV-monoinfected and HIV/HCV-coinfected individuals from the GEHEP-011 cohort [inclusion criteria: 1] LS prior to treatment=9.5 kPa; 2] SVR with DAA-based therapy; 3] Available LS measurement at SVR]. LS was evaluated pre-treatment, at RVS and then annually until 60 months. The following LS categories were considered: ≤7.2 kPa; 7.3-9.4 kPa; 9.5-13.9 kPa; ≥14-20.9 kPa; and ≥21 kPa. LS normalisation was defined as attaining LS values ≤7.2 kPa after SVR.

Results: 1116 patients were included. 677 (61%) lived with HIV. 675 (61%) had LS≥14 kPa prior to treatment; 422 (62%) HIV/HCV-coinfected and 253 (58%) HCV-monoinfected individuals (p=0.016). At SVR time-point, 468 (42%) patients showed LS=14 kPa (HIV/HC 289 [43%] vs. 179 [41%], p=0.527). Median (Q1-Q3) LS at each time point was: pre-treatment 16.7 (11.8-26.6) kPa, SVR 11.9 (7.9-20.2) kPa, 12 months post-SVR 10.3 (7.0-17.7) kPa, 24 months post-SVR 9.4 (6.6-16.4) kPa, 36 months post-RVS 9.7 (6.7-15.4) kPa, 48 months post-RVS 10.4 (7.0-16.5) and 60 months post-RVS 10.0 (7.0-16.8) kPa (p<0.001). Median (Q1-Q3) LS between first and last LS measurement was 29 (13-48) months. Taking into account the first and the last LS measurement, 789 (71%) individuals improved from 1 or more lower LS categories, in 288 (26%) there was no change, and 39 (3%) worsened. More specifically, 317 (27%) achieved LS normalization, 178 (26%) HIV/HCV-coinfected patients vs. 139 (32%) HCV-monoinfected individuals (p=0.052).

Conclusion: After SVR, LS decreases significantly. This reduction is quantitatively greater at the time of SVR and the following year. In a large majority of patients, this improvement also leads to a shift to lower LS categories. Specifically, more than a quarter of individuals achieved LS normalization during the follow-up. However, in one third of patients, LS does not change or even increases.

608 METABOLIC PROFILE ASSOCIATED WITH HVPG CHANGE AFTER DAA IN HCV Pts WITH CIRRHOSIS

Ana Vírseca Berdices1, Juan Berenguer2, David Rojo3, Juan González-García4, Oscar Brochado Kith1, Amanda Fernández-Rodriguez2, Daniel Sepulveda Crespo1, Cristina Diez2, Victor Montanón1, Leire Pérez-Latorre1, Rafael Micán6, Teresa Aldáiz Echevarría1, Coral Barbás1, Salvador Resino3, María Á. Jiménez-Sousa1 * Institute of Health Carlos III, Majadahonda, Spain, 2Hospital Universitario Gregorio Marañón, Madrid, Spain, 3Centre for Metabolomics and Bioanalysis, Madrid, Spain, 4La Paz University Hospital, Madrid, Spain, 5Institute of Salud Carlos III, Madrid, Spain, 6La Paz University Hospital, Majadahonda, Spain, Centro Nacional de Microbiología, Madrid, Spain

*Presented at CROI by a nonauthor colleague

Background: Hepatic venous pressure gradient (HVPG) is currently the best available predictor of liver-related outcomes in cirrhotic patients. In response to direct-acting antivirals (DAAs) therapy, patients who experience a decrease in HVPG considerably reduce liver complications and higher survival. This study aimed to assess the metabolic changes associated with the variation in HVPG from the start of DAA therapy until 48 weeks after effective DAAs therapy in patients with advanced HCV-related cirrhosis.

Methods: We performed a multicenter prospective study in 31 patients with advanced cirrhosis and clinically significant portal hypertension (HVPG≥10 mmHg) (8 HCV-monoinfected and 23 HIV/HCV-coinfected). Patients were assessed at baseline and 48 weeks after DAAs therapy completion. Metabolomics analysis was carried out by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Inflammatory plasma biomarkers were analyzed by ProcartaPlex Immunoassays. The statistical analysis was performed by partial least squares discriminant analysis (PLS-DA) and generalized linear mixed-effects models, correcting for multiple testing.

Results: The PLS-DA analysis showed that the two sample sets, baseline and 48 weeks after DAA therapy, clearly separated according to the metabolomic profiles for GC-MS and LC-MS (permutation < 0.05). Thirty compounds in GC-MS and 64 in LC-MS had a VIP score ≥2. Of them, we found a direct association between the change in plasma levels of 2, 3-butandiol (AMR=1.15; p≤0.001; q=0.010), taurocholic acid (AMR=1.20; p=0.001; q=0.001) and N-gamma-glutamyl-S-allylcysteine (AMR=1.01; p=0.019; q=0.090) and the HVPG change. On the contrary, an indirect association was observed between the plasma levels of tartaric acid and the variation of HVPG (AMR=0.96; p=0.020; q=0.091). Finally, we found direct association between changes in taurocholic acid and inflammatory plasma biomarkers (IL6, IL8, IL1RA, IP10 and sICAM1).

Conclusion: Plasma metabolomic profile changed along with the HVPG from baseline to 48 weeks after completing DAs therapy in patients with advanced HCV-related cirrhosis. Specifically, increased plasma levels of tartaric acid and decreased of 2,3-butandiol, taurocholic acid, and N-gamma-glutamyl-S-allylcysteine were associated with decreases in HVPG. In addition, the change in plasma taurocholic acid level was directly associated with inflammatory plasma biomarkers.

609 PREVALENCE AND FACTORS ASSOCIATED WITH NAFLD IN PEOPLE WITH HIV

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Background: Non-alcoholic fatty liver disease (NAFLD) in people living with HIV (PLHIV) may be more common than in the general population. Screening recommendations for NAFLD in PLHIV are based on guidelines for the general population, not considering HIV-specific factors contributing to NAFLD. In this study, we assessed the prevalence of liver steatosis and fibrosis in PLHIV and explored associations with demographic-, metabolic-, environmental- and HIV-specific factors, including antiretroviral therapy (ART).

Methods: In the 2000HIVH study (clinicaltrials.gov NCT039948435), 1895 virally suppressed PLHIV were included in 4 Dutch HIV treatment centers. Transient elastography was performed for the assessment of liver steatosis (controlled attenuation parameter, CAP) and fibrosis (liver stiffness measurement, LSM). Demographics, metabolic comorbidities, laboratory assessments including lipid profile and liver function tests, HIV-characteristics, and current and cumulative exposure to ART regimens, were tested in univariable (demographic factors) and multivariable (other factors) logistic regression models for association with steatosis and fibrosis.

Results: Data from 1075 PLHIV showed steatosis in 47.5% [95% CI: 44.4 – 50.6] and fibrosis in 8.8% [7.1 – 10.6]. Age (per decade, odds ratio (OR) 1.49, 95% CI [1.33-1.66], P-value <0.001, and OR 1.12 [0.93-1.34], P-value = 0.356, respectively) and subcutaneous fat layer thickness (E 25 mm² > 25 mm², OR 5.16 [3.90-6.83], P-value <0.001, and OR 2.72 [1.69-4.39], P-value <0.001, respectively) were significantly associated with steatosis and fibrosis and included in subsequent regression models as covariates. Traditional metabolic risk factors, e.g. diabetes mellitus type 2 (adjusted OR [aOR] 1.99 [1.02-3.71], P-value = 0.043, and aOR 3.29 [1.64-6.62], P-value = 0.001, respectively) were most strongly associated with both steatosis and fibrosis. In addition, steatosis was associated with current CD4+ and CD8 + T cell counts, cumulative exposure to integrase strand transfer inhibitors (INSTI) in general and raltegravir specifically, and to the nucleoside analogue stavudine.

Conclusion: Liver steatosis and fibrosis affect nearly one in two and one in ten PLHIV in this cohort, respectively. NAFLD was most strongly associated with traditional NAFLD risk factors. Of HIV-specific factors, only exposure to
stavudine, INSTI, and current CD4+ and CD8+ T cell counts were associated to steatosis.

### 610 LIVER STEATOSIS AND FIBROSIS IN WOMEN WITH HIV BY INTEGRASE INHIBITOR USE

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1Emory University, Atlanta, GA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3Georgetown University, Washington, DC, USA, 4University of Mississippi Medical Center, Jackson, MS, USA, 5University of Miami, Miami, FL, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 7Stroger Hospital of Cook County, Chicago, IL, USA, 8State University of New York Downstate Medical Center, Brooklyn, NY, USA, 9Albert Einstein College of Medicine, Bronx, NY, USA

**Background:** Nonalcoholic fatty liver disease is prevalent in persons with HIV and can lead to hepatocellular fibrosis. Integrase strand-transfer inhibitors (INSTIs), first-line agents in antiretroviral therapy (ART), are associated with increased body mass index (BMI), particularly in women with HIV (WWH). We evaluated hepatic steatosis and fibrosis in WWH who did and did not switch to INSTIs.

**Methods:** We used clinical and FibroScan data collected between 2014-2018 in virally-suppressed WWH enrolled in the Liver Disease and Reproductive Aging substudy of the Women’s Intercagency HIV Study. WWH who switched to or added an INSTI to ART if they had complete data and a FibroScan obtained post-switch and compared to women on non-INSTI ART (Control). Follow-up time was defined as time since switch visit (or comparable visit in Controls). Outcomes included differences between INSTI and Control group estimates of hepatic steatosis via controlled attenuation parameter (CAP≥248 dB/m), fibrosis via liver stiffness (LS≥7 kPa), and steatohepatitis with significant disease activity and fibrosis via FibroScan-aspartate aminotransferase scores (FAST ≥0.67). Adjusted regression and mixed-effects models compared each outcome by group.

**Results:** 257 WWH (123 INSTI, 134 Control) were included. Overall, mean age was 50 years (SD 8), 74% were Non-Hispanic Black, BMI was 32 (8) kg/m², CD4 count was 796 (305) cells/mm³. WWH who switched to INSTIs had a 3.7 greater odds of having hepatic steatosis by 1 year compared to non-INSTI Controls (Table 1), but this difference was not seen at later periods of follow up. The model-adjusted difference between WWH switching to INSTIs vs Controls within 1 year was +1.62 KPa (95% CI 0.24, 2.99) for LS and +0.07 (0.01, 0.33) for FAST scores. However, there was little difference between groups in the odds of having fibrosis and only 4(1.6%) WWH had steatohepatitis by FAST score at any time-point.

**Conclusion:** WWH switching to INSTIs had a greater odds of having hepatic steatosis but not fibrosis within the 1st year of follow-up compared to women on non-INSTI ART, possibly reflecting early BMI gain with INSTIs. LS and FAST scores in WWH switching to INSTIs were minimally higher at 1 year, but the clinical significance is unclear. A larger study with longitudinal assessments of hepatic steatosis and fibrosis measures is warranted. Patients starting INSTIs need close monitoring of metabolic changes and low thresholds to pursue noninvasive liver fibrosis testing.

**Adjusted model estimates of hepatic steatosis and fibrosis differences between groups.**

**Table 1. Adjusted model estimates of hepatic steatosis and fibrosis differences between groups**

<table>
<thead>
<tr>
<th>Continuous outcomes</th>
<th>Categorical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Difference between INSTI and Control at follow-up time</strong></td>
<td><strong>Odds Ratio</strong></td>
</tr>
<tr>
<td><strong>since switch visit (90% CI)</strong></td>
<td><strong>CAP (dBi/mm)</strong></td>
</tr>
<tr>
<td><strong>&lt; 1 year</strong></td>
<td>24.26 (20.48, 28.09)</td>
</tr>
<tr>
<td><strong>1 to 2 years</strong></td>
<td>5.01 (3.82, 6.41)</td>
</tr>
<tr>
<td><strong>≥ 3 years</strong></td>
<td>17.78 (13.40, 22.05)</td>
</tr>
</tbody>
</table>

**Conclusion:** MAFLD seems a sexual dimorphic disease in PWH. Despite having lower rates of MAFLD, women with HIV have higher incidence of significant liver fibrosis compared to men, especially after 50 years of age. Future studies should target adequate consideration of sex differences in clinical investigation of MAFLD to fill current gaps and implement precision medicine for PWH.

### 611 SEX DIFFERENCES IN THE ASSOCIATION OF HIV WITH METABOLIC-FATTY LIVER DISEASE

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1IAGG University Health Centre, Montreal, QC, Canada, 2University of Modena and Reggina Emilia, Modena, Italy, 3IAGG University, Montreal, QC, Canada, 4University of Palermo, Palermo, Italy

**Background:** People with HIV (PWH) are at high risk for metabolic dysfunction associated fatty liver disease (MAFLD). In the general population, sex differences seem to exist in frequency and severity of MAFLD, with higher prevalence of MAFLD in men, but higher incidence of liver fibrosis in women. Less is known about sex differences in MAFLD and liver fibrosis in the setting of HIV infection.

**Methods:** This was a multicenter cohort study including consecutive PWH who underwent screening for MAFLD and liver fibrosis by liver stiffness measurement (LSM) with associated controlled attenuation parameter (CAP). MAFLD was defined as the presence of hepatic steatosis, diagnosed as CAP>270 dB/m, plus any among type 2 diabetes, overweight (BMI>25 kg/m²) or two other metabolic abnormalities. Significant liver fibrosis was diagnosed as LSM>8 kPa. Incidence of MAFLD and significant liver fibrosis was assessed through survival analysis.

**Results:** 1359 PWH (25% females, 70% HIV mono-infected) were included. Prevalence of MAFLD at baseline was lower in women than in men with HIV (17.2% vs. 24.3%, p=0.013). Conversely, there was no difference in prevalence of liver fibrosis (10.7% vs. 13.4%). Women with MAFLD were more frequently of black ethnicity (48% vs. 14%, p<0.001), had lower ALT (26.4+20.4 vs. 33.4+22.5; p=0.035), higher HDL cholesterol (1.46+0.57 vs. 1.11+0.33; p<0.001), lower triglycerides (1.69+0.96 vs. 2.47+2.63; p=0.035) compared to men with MAFLD. 485 of these PWH were followed for a median of 3.5 years. Incidence of MAFLD was similar between women and men with HIV. However, incidence of liver fibrosis was higher in women compared to men with HIV (7.0 per 100 person-years (PY) vs. 5.9 per 100 PY; p=0.035) (Figure 1). The higher incidence of significant liver fibrosis occurred particularly after the age of 50 years. Multivariable Cox regression analysis and after adjusting for age, presence of MAFLD (adjusted hazard ratio (aHR) 3.3, 95% CI 2.0-5.6) and female sex (aHR 2.2, 95% CI 1.3-3.5) were independent predictors of developing significant liver fibrosis while CD4 cell count was protective (aHR 0.99, 95% CI 0.99-0.99).

**Conclusion:** MAFLD seems a sexual dimorphic disease in PWH. Despite having lower rates of MAFLD, women with HIV have higher incidence of significant liver fibrosis compared to men, especially after 50 years of age. Future studies should target adequate consideration of sex differences in clinical investigation of MAFLD to fill current gaps and implement precision medicine for PWH.

### 612 NAFLD AND ITS COMBINATION WITH NASH PREDICT DM DEVELOPMENT IN PEOPLE WITH HIV

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**Background:** We investigated the association of non-alcoholic fatty liver disease (NAFLD) plus or minus a concurrent diagnosis of non-alcoholic steatohepatitis (NASH) with incident diabetes mellitus (DM) among Thai people living with HIV (PLWH).

**Methods:** This prospective study analysed PLWH aged ≥18 years, on stable antiretroviral therapy (ART), in a long-term cohort in Bangkok, Thailand. Liver
stiffness (LS) and controlled attenuated parameter (CAP) values were collected from FibroScan. NAFLD was defined as CAP >248 dB/m, whereas NASH was defined as FibroScan-AST (FAST) score >0.7. Baseline was defined as the first FibroScan date. PLWH with hepatitis B or C virus infection, those with excessive alcohol consumption or with DM diagnosed prior to baseline were excluded. Cox proportional hazard models were used to investigate the association of NAFLD and NASH with incident DM. We also investigated the association of NAFLD with DM at baseline with incident NASH.

Results: Among 847 eligible PLWH, the median age at baseline was 46 (IQR 39-52) years (43% female). Median baseline CD4 was 588 (IQR 443-759) cells/mm³ and 90% had HIV RNA <50 copies/mL. Median CAP value and FAST score was 215 (IQR 184-254) dB/m and 0.22 (IQR 0.12-0.43) respectively. 28% (235/847) and 15% (116/781) had NAFLD and NASH at baseline, respectively. During a median follow-up time of 3.3 (IQR 2.7-3.6) years, 28 developed DM (incidence rate=11.0% (95%CI 7.6-15.9) per 1000 person-years). Baseline NAFLD was associated with an increased risk of incident DM (hazard ratio [HR]: 2.8, 95%CI 1.3-6.4) after adjusting for age, sex, family history of DM, ART duration, and didanosine exposure, and time-updated BMI, hypertension and dyslipidemia. Combined NAFLD and NASH at baseline increased the risk of incident DM (HR: 3.1, 95%CI 1.1-9.3). Figure 1A and 1B show the probability of incident DM stratified by NAFLD and NASH status at baseline. Baseline NASH alone showed a non-significant but elevated risk of incident DM (HR: 1.8, 95%CI 0.7-4.6). In a separate analysis including DM at baseline (but excluding NASH at baseline), NAFLD with DM at baseline was associated with incident NASH (HR: 2.5, 95%CI 1.1-6.0).

Conclusion: NAFLD alone or combined with NASH strongly predicts DM development in PLWH, highlighting the need for DM risk assessment and management in PLWH with NAFLD. Further mechanistic studies investigating the underlying metabolic associations of NAFLD or NASH and DM development in PLWH are warranted.

614 CONTROLLED ATTENUATION PARAMETER IS A VISCERAL ADIPOSEITY MARKER IN HIV-RELATED NAFLD

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1McGill University, Montreal, QC, Canada, 2McGill University Health Centre, Montreal, QC, Canada, 3University of Modena and Reggio Emilia, Modena, Italy

Background: Fat alterations are frequent in people with HIV (PWH) and predict worse cardiometabolic outcomes. Visceral adipose tissue (VAT) is a hormonally active tissue critically contributing to obesity-related disorders and associated with ectopic fat accumulation in the liver. We aimed to investigate HIV-associated nonalcoholic fatty liver disease (NAFLD) diagnosed by controlled attenuation parameter (CAP) as a marker of visceral adiposity.

Methods: We conducted a prospective pilot study (ClinicalTrials.gov NCT05359471) of HIV mono-infected patients undergoing metabolic characterization and paired CAP by transient elastography with dual-energy X-ray absorptiometry (DEXA) scan. NAFLD was defined as CAP ≥285 dB/m, in absence of alcohol abuse. Excess visceral adiposity was defined as VAT >1.32 Kg. Pairwise correlation, area under the curve (AUC) and logistic regression analysis were employed to study the association between VAT and CAP.

Results: 30 patients (90% male, mean age 49, mean BMI 30, mean waist circumference 101cm, 50% with NAFLD) were included. When compared to those without excess VAT, PWH with excess VAT were older, had longer duration of HIV infection, had higher BMI and waist circumference. They also had more history of cardiovascular events, higher triglycerides and LDL cholesterol. CAP was higher in PWH with excess VAT (p=0.001). CAP positively correlated with all visceral fat measurements by DEXA, including VAT (r=0.650, p=0.001), VAT/body weight ratio (r=0.565, p=0.001) and fat mass (r=0.390, p=0.033). Both BMI and waist circumference showed correlation with VAT and fat mass, but not with VAT/body weight ratio (see Figure). After adjusting for duration of HIV infection (aOR 1.01 per year, 95% CI 0.91-1.12; p=0.921), BMI (aOR 1.77, 95% CI 0.74-4.23; p=0.202) and waist circumference (aOR 0.91 per cm, 95% CI 0.68-1.21; p=0.509), CAP remained the only independent predictor of excess VAT (aOR 1.05 per dB/m, 95% CI 1.01-1.10; p=0.036). The AUC analysis determined CAP had excellent performance to diagnose excess VAT (AUC 0.92, 95% CI 0.81-1.00), higher than BMI (AUC 0.83, 95% CI 0.68-0.99) and waist circumference (AUC 0.81, 95% CI 0.65-0.97). The optimized CAP cut-off to diagnose excess VAT was 266 dB/m, with a sensitivity of 89.3% and a specificity of 84.6%.
Conclusion: NAFLD diagnosed by CAP is associated with VAT in PWH independently of anthropometric measures of obesity. CAP could be used as a diagnostic marker of visceral adiposity in the practice of HIV medicine.

Correlation matrix with heatmap based on correlation coefficient analysis. Significant values (p<0.05) are bolded.

Mean annual percent changes (95% Confidential Interval) of CAP and metabolic parameters

Table 1: Mean annual percent changes (95% Confidence Interval) of CAP and metabolic parameters

Figure 1 Receiver operator characteristic (ROC) curve for Fatty Liver Index (FLI, solid line) and Hepatic Steatosis Index (HSI, dashed line) for the detection of severe liver steatosis in the whole study population.
617 RISK FACTORS FOR LIVER FIBROSIS PROGRESSION IN HIV: A MULTI-CENTER LONGITUDINAL STUDY
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1University of Modena and Reggio Emilia, Modena, Italy, 2University of Brescia, Brescia, Italy, 3University of Bonn, Bonn, Germany, 4Azienda Ospedaliero Universitaria Policlinico di Modena, Modena, Italy, 5McGill University, Montreal, QC, Canada, 6McGill University Health Centre, Montreal, QC, Canada, 7University of Modena and Reggio Emilia, modena, Italy, 8University Hospital Bonn, Bonn, Germany

Background: People with HIV (PWH) are at high risk for metabolic dysfunction-associated fatty liver disease (MAFLD). Liver fibrosis (LF) is the most significant predictor of liver disease progression and mortality. We aimed to investigate the effect of MAFLD and antiretroviral exposure on LF progression in PWH.

Methods: This was a longitudinal study of three large prospective cohorts of PWH in Italy, Germany and Canada. Patients with at least two transient elastography with controlled attenuation parameters (CAP) exams were included. LF progression was defined as development of significant LF (defined as liver stiffness >8 kPa), or transition to cirrhosis (defined as liver stiffness >13 kPa for those with liver stiffness >8 but <13 kPa at baseline). MAFLD was defined according to Eslam criteria: presence of hepatic steatosis (CAP>24 dB/m), plus any among type 2 diabetes, overweight (BMI >25 Kg/m2) or defined according to Eslam criteria: presence of hepatic steatosis (CAP>24 dB/m), plus any among type 2 diabetes, overweight (BMI >25 Kg/m2) or two other metabolic abnormalities. Other longitudinal predictors included co-infection with HBV or HCV, weight gain (WG), defined as a 5% BMI increase in two consecutive visits, and current exposure to ART classes. A multi-state Markov model was used to describe the process in which PWH moved through the next LF state. Cox regression model was used to identify predictors for LF progression event.

Results: A total of 1183 PWH were included (median age 52.9 years, 77% males, median duration since HIV diagnosis 18 years). Prevalence of MAFLD was 46.8%. Coinfections with HBV and HCV were present in 3.6% and 21%, respectively. At baseline, liver stiffness was <8 kPa in 85.6%, 8.1-12.9 kPa in 8.6%, and >13 kPa in 5.7% of PWH. During a median follow-up period of 2.5 years, a minimum of two and maximum of six yearly LF assessments were performed. In Markov model, WG was positively associated with progression of LF (OR=3.107, 95% CI 1.588, 6.078) while it prevented LF regression (OR=0.304, 95% CI 0.037, 2.514).

The incidence rate of LF progression was 3.4 per 100 persons-year. Comparing 128 (9.6%) LF progressors with 1212 (90.4%) of non LF progressors, significant differences included mean BMI (26.3 vs 24.5), duration of HIV (16.7 vs 18.6 yrs), MAFLD (66.7 vs 48.3), HBV co-infection (7.8 vs 3.5%), ALT (36 vs 21 IU) and WG (32.4 vs 21.9%). On multivariable analysis, predictors of LF progression were WG and MAFLD (see Table).

Conclusion: LF progression occurs in a significant proportion of PWH. Its main drivers include metabolic health variables, while ART exposure does not seem to impact LF progression. Cox regression analysis of the liver fibrosis progression

<table>
<thead>
<tr>
<th>Predictor</th>
<th>HR (95% CI)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Current exposure to WG</td>
<td>1.47 (0.61 - 3.22)</td>
<td>0.306</td>
</tr>
<tr>
<td>Current exposure to TAF</td>
<td>0.85 (0.38 - 1.97)</td>
<td>0.691</td>
</tr>
<tr>
<td>Current exposure to NEAT</td>
<td>0.63 (0.32 - 1.28)</td>
<td>0.174</td>
</tr>
<tr>
<td>Current exposure to IF</td>
<td>1.23 (0.64 - 2.33)</td>
<td>0.490</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.09 (0.43 - 2.71)</td>
<td>0.805</td>
</tr>
<tr>
<td>Age at baseline</td>
<td>0.99 (0.89 - 1.09)</td>
<td>0.996</td>
</tr>
<tr>
<td>Necro-Grade 2.00</td>
<td>0.99 (0.57 - 1.77)</td>
<td>0.935</td>
</tr>
<tr>
<td>Years since HIV diagnosis</td>
<td>1.05 (0.93 - 1.10)</td>
<td>0.094</td>
</tr>
<tr>
<td>Chronic Hepatitis B virus infection</td>
<td>2.09 (0.36 - 1.27)</td>
<td>0.232</td>
</tr>
<tr>
<td>Chronic Hepatitis C virus infection</td>
<td>1.09 (0.43 - 2.57)</td>
<td>0.936</td>
</tr>
<tr>
<td>MAFLD</td>
<td>2.50 (1.08 - 5.89)</td>
<td>0.030</td>
</tr>
<tr>
<td>BMI gain &gt;5%</td>
<td>2.94 (1.32 - 6.55)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

618 INCIDENCE OF NON-HEPATIC CANCERS IN HBV, HCV, AND HBV/HCV INFECTED PERSONS
Adeel Butt1, Peng Yao2, Obaid Shaikh2
ERCHIVES (Electronically Retrieved Cohort of HCV Infected Veterans)
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Background: Hepatitis B and C (HBV, HCV) are among the leading causes of hepatocellular carcinoma (HCC) worldwide. Emerging data suggest that they may also be associated with hepatic and non-hepatic cancers. Co-infection may confer a higher risk than mono-infection with either virus. We sought to determine the incidence rate of Intrahepatic Cholangiocarcinoma (IHC), Non-Hodgkin lymphoma (NHL), Pancreatic cancer (PC), Colorectal cancer (CRC), and Gastric cancer (GC) among Veterans with these infections in ERCHIVES, a national cohort of HCV infected Veterans and matched HCV-uninfected controls.

Methods: Among the participants of ERCHIVES, we first identified Veterans with HBV and HCV mono-infection and HBV/HCV coinfection. We excluded those with a history of any cancer of interest, those with missing data to calculate FIB-4 score, and those with HIV coinfection. We then propensity-score matched each HBV infected person with an HCV and a HBV/HCV infected person (1:1:1 matching). We calculated the incidence rate per 1,000 person-years of follow-up for IHC, NHL, PC, CRC, and GC overall, and stratified by presence of cirrhosis at baseline. Cancer diagnoses were based on presence of respective ICD-9/ICD-10 (clinical modification) codes.

Results: Among 818,318 participants of ERCHIVES, we identified 1,525 HBV mono-infected, 181,620 HCV mono-infected, and 1,380 HBV/HCV infected persons. The final propensity-score matched dataset included 990 HBV mono-infected, 1,374 HCV mono-infected, and 1,375 HBV/HCV infected persons. The incidence rates per 100-persons years were numerically highest for the cancers of interest among HBV mono-infected persons. However, the rates were not statistically significantly different among any of the comparison groups except for a higher incidence of PC among HBV mono-infected compared with HCV mono-infected persons.

Conclusion: HBV infection is associated with a numerically higher incidence rates for IHC, NHL, PC, CRC, and GC. However, among the demographically and clinically matched HBV, HCV, and HBV/HCV coinfected persons, the rates are not statistically significantly different except higher PC rate HBV compared with HCV mono-infected persons.

Table. Incidence rate per 1,000 person-years of follow-up of selected cancers by infection status.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Incidence Rate per 1,000 Person-Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular Cholangiocarcinoma</td>
<td>0.047 (0.028, 0.105)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>1.030 (0.772, 1.358)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>0.936 (0.550, 1.595)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>0.887 (0.681, 1.152)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>0.370 (0.291, 0.468)</td>
</tr>
</tbody>
</table>

619 CONTRIBUTION OF ALCOHOL USE IN HVC/HCV INFECTION TO ALL-CAUSE MORTALITY
Adam Trickey1, Lei Zhang3, Suzanne M. Ingle4, Anders Boyd1, Sophie Grabar4, Inmaculada Jarrin1, Niels Obel5, M. John Gill6, Robert Zangerle7, Andri Rauch7, Christopher T. Rentsch1, Derek D. Satre2, Heidi Crane1, Jonathan Ac Sterne1, Linda Wittkop1

Antiretroviral Therapy Cohort Collaboration (ART-CC)
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Background: Among persons with HIV (PWH), both alcohol use and having hepatitis C virus (HCV) are separately associated with increased morbidity and mortality. We aimed to investigate whether the association between alcohol use and mortality among PWH is modified by having HCV.

Methods: Data were combined from European and North American cohorts contributing to the Antiretroviral Therapy Cohort Collaboration (ART-CC) of adult PWH who started antiretroviral therapy (ART) between 2001-2017. Baseline was date of ART initiation and PWH were followed for mortality. Self-reported current alcohol use data, collected in diverse ways between cohorts, were harmonised to give a value of grams alcohol/day. HCV antibody and/
or HCV RNA tests when available were used to define HCV status at baseline. Effect modification by baseline alcohol use (0, 0.1-20.0, >20.0 grams/day) and HCV status was assessed using interaction terms in multivariable Cox models, adjusted for ART start year category, HIV acquisition group, gender, prior AIDS status, age, CD4 count cells/μL, and log HIV-1 RNA copies/mL.

**Results:** Of 58,769 PWH, 21% were women and the median age at ART initiation was 40 years (interquartile range: 32-49). 25,711 (51%) self-reported alcohol use of 0g/day, 23,974 (41%) 0.1-20.0g/day, and 5,064 (9%) >20.0g/day, and 4,799 (8%) had HCV at baseline. The mortality rate per 1,000 person-years was highest among PWH with HCV (21.6) than without HCV (6.2). Among PWH without HCV, there was a J-shaped relationship between mortality and alcohol use with adjusted hazard ratios (aHRs) of 1.21 (95%CI: 1.11-1.32) for 0g/day and 1.87 (1.65-2.12) for >20.0g/day compared with 0.20-20.0g/day (see table). The J-shaped pattern was not seen for those with HCV (treatment p-value < 0.001), with aHRs 0.99 (0.85-1.15) for 0g/day and 1.71 (1.39-2.10) for >20.0g/day compared with 0.20-20.0g/day.

**Conclusion:** Among PWH without HCV, mortality was higher in both persons who reported not drinking and persons who reported drinking heavily compared with persons who reported drinking moderately. Among PWH without HCV, mortality was higher only in persons who reported drinking heavily, potentially due to differing reasons for not drinking between PWH with and without HCV (e.g. illness).

Adjusted mortality hazard ratios (with 95% confidence intervals) for alcohol use categories, stratified by HCV RNA status.

<table>
<thead>
<tr>
<th>Alcohol use (grams per day)</th>
<th>HCV-negative</th>
<th>HCV-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (deaths)</td>
<td>aHR (95% CI)</td>
<td>n (deaths)</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0g</td>
<td>2701 (1706)</td>
<td>1.19 (1.13-1.27)</td>
</tr>
<tr>
<td>0.1-20.0g</td>
<td>2806 (1708)</td>
<td>1.32 (1.29-1.36)</td>
</tr>
<tr>
<td>&gt;20.0g</td>
<td>4413 (294)</td>
<td>1.87 (1.79-2.11)</td>
</tr>
</tbody>
</table>

620 SYNDROME OF CHRONIC VIRAL INFECTIONS ON THE RISK OF END-STATE RENAL DISEASE

Dahn Jeong, Stanley Wong, Jason Wong, Jean Damascene Makuzza, Geoffrey McKee, Héctor A. Velásquez García, Zahid A. Butt, Prince A. Adu, Mawuena Binka, Sofia Bartlett, Amanda Yu, Maria Alvarez, Mel Krajden, Naveed Z. Janjua

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**Background:** Thanks to modern HIV therapy, people living with HIV now have a far longer lifespan; however, people aging with HIV are more likely to develop chronic diseases. Syndemic of viral infections is also associated with increased risk of end-stage renal disease (ESRD). Additionally, hypertension and diabetes are important risk factors for ESRD. We assessed the impact of HIV, HCV, and HIV mono-, co- and triple infections on incident ESRD, in people with and without hypertension or diabetes.

**Methods:** All people who were tested in British Columbia (BC) between 1990 and 2015 for HIV, HCV, and HIV are included in the BC Hepatitis Testers Cohort and linked to administrative health databases. Individuals tested for all three infections were followed from the date of their last test until 1) incident ESRD 2) death or 3) 2021/03/31. We adjusted for sex, birth year, ethnicity, material/social deprivation, alcohol use, drug dependence, major mental illness, opioid agonist therapy and injection drug use, and estimated subdistributional hazard ratios (sHRs) for incident ESRD with Fine-Gray competing risk models. The impact of surveillance for ESRD is crucial for the aging population with viral infections, especially among those with hypertension or diabetes.

**Figure:** Cumulative incidence curves of incident ESRD among individuals with viral infections; table shows ESRD incidence rates per 1,000 person-years (95% confidence interval)

621 LIVER NEUTROPHILS EXPRESS STRONGER PD-L1 SIGNALING IN HIV/HBV COMPARED TO HBV ALONE

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1Massachusetts General Hospital, Boston, MA, USA, 2University of Zambia, Lusaka, Zambia, 3Queen Mary University of London, London, United Kingdom, 4Center for Infectious Disease Research in Zambia, Lusaka, Zambia, 5University of California Los Angeles, Los Angeles, CA, USA

**Background:** HIV coinfection accelerates the natural history of hepatitis B infection (HBV); however, precise mechanisms for this are not fully understood, in part because of the site of infection – the liver – is difficult to access. Further, patients with HIV/HBV coinfection may experience robust anti-HBV immune responses when starting antiretroviral therapy. In Zambia, where HIV/HBV coinfection is common, we established capacity for liver fine needle aspiration (FNA), a less invasive approach than core biopsy, which is more acceptable to patients for longitudinal sampling. Our aim is to understand how immune cells are modulated in the setting of HIV/HBV coinfection and mechanisms for HBV functional cure in coinfection.

**Methods:** Treatment-naïve adults with either HIV/HBV coinfection or treatment-eligible chronic HBV monoinfection were enrolled and underwent liver FNA at University Teaching Hospital in Lusaka. Liver cells were captured and analyzed using the HIV single cell RNA sequencing approach (Honeycomb Biotechnologies). This analysis included liver FNAs from 12 patients (6 each) with HBV monoinfection and HIV/HBV coinfection.

**Results:** The median age of analyzed patients was 31 years (range: 20-45) and were female (2 in each group). Coinfected patients had median peripheral CD4 count of 225 cells/mm³ (range: 69-999). Across the patients, transcriptome profiling of a total of 8,785 immune cells from FNA samples showed significant differences in gene expression by HIV status across a range of immune cell types. HIV/HBV coinfection was associated with increased expression of interferon-stimulated genes (ISGs). Notably, neutrophils captured in the sample overexpressed PD-L1 compared to other immune cells, and this correlated with increased expression of PD-1 in CD8 T cells. Clustering of neutrophils revealed 3 subclusters, including one that strongly expressed both PD-L1 and ISGs. In HIV/HBV coinfection, neutrophil PD-1/PD-L1 signaling was significantly stronger compared to HBV monoinfection.
Conclusion: These data support neutrophil participation in the exhaustion and suppression of antiviral immune responses, a phenotype that is exacerbated in the setting of chronic HIV/HBV coinfection. This could represent a potential mechanism for T cell exhaustion and increased HIV chronicity in persons living with HIV.

622 YAP-MEDIATED HEPATOCYTE DAMAGE CONTRIBUTES TO LIVER FIBROSIS WITH HIV-RELATED INJURY
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Background: HIV infection is known to accelerate the progression of liver fibrosis. In addition, Yes-associated protein (YAP) is a key regulator of cell proliferation and organ size. Recent work has shown that both the YAP and PI3K/ AKT pathways are critical to hepatic fibrogenesis. Lysophosphatidic acid (LPA) and LPAR1 is involved in the fibrotic pathogenesis of hepatocellular carcinoma. However, their contribution to these processes during and after HIV-induced liver injury has not been fully explored. In this study, we sought to determine the role of YAP in modulating liver injury and recovery in the context of HIV infection.

Methods: We utilized liver specimens derived from an HIV-infected humanized murine model with and without antiretroviral therapy (ART). Further, a spheroid system and other coculture techniques allowed for further analysis of liver cell lines, primary human hepatocytes (pHsHs), and primary hepatocellular stellate cells (pHSCs) exposed to HIV. Serum samples from HIV patients before and after antiretroviral treatment (ART) were also analyzed via ELISA.

Results: The YAP pathway components CYR61 and CTGF were increased within the livers of a humanized mouse model which demonstrated histologically worsened fibrosis after HIV infection. ART was effective partially abrogating these effects. In vitro analysis confirmed that YAP-related and profibrogenic genes were upregulated within pHSCs and Huh7 cells exposed to HIV. An HIV-activated profibrotic program was dependent on hepatocyte-derived YAP within both Huh7s and pHSCs. Treatment of Huh7s and HSCs with verteporfin significantly abrogated the effects of HIV on both cell types. Moreover, when multiple HIV proteins were used as exposure agents among Huh7 cells, only GP120 was found to be responsible for activating this profibrotic program. Additionally, serum samples from treatment-naive patients were analyzed via ELISA and demonstrated elevated levels of circulating YAP-related proteins in the context of HIV infection compared to uninfected controls. This elevation, while diminished, persisted even after 6 months of ART. Lastly, experiments utilizing siRNAs, overexpression of LPAR1, and small molecule inhibitors implicated PI3K and AKT as critical contributors to a profibrotic mechanism linking HIV infection to YAP activation.

Conclusion: This work suggests that HIV-related liver fibrosis and repair depends on hepatocyte injury and hepatic stellate cell activation via the YAP/ LPAR/PI3K/AKT pathway.

623 CHRONIC LIVER INFLAMMATION AND USE OF CONTEMPORARY ART AMONG PERSONS LIVING WITH HIV
Ashley Roen
RESPOND University College London, London, United Kingdom

Background: Whilst use of some older antiretroviral drugs (ARVs) are associated with chronic liver enzyme elevation (cLEE), the impact of newer ARVs remain unknown.

Methods: Using RESPOND data, we included individuals who started an ARV to which they had not been previously exposed after the 2012 study baseline. ARVs considered were INSTIs: DTG, ETV/c, RAL, BIC; NNRTIs: RVP and EFV; NRTIs: TAF and TDF; PIs: DRV/r, ATV (see figure for abbreviations). Eligible Individuals had an HIV-RNA, CD4, and all alanine transaminase (ALT) measurements normal one year before baseline. The primary outcome was first cLEE: ALT elevations > than the upper limit of normal (males >50 IU/L, females >35 IU/L) at ≥2 visits over ≥6 months and < 2 years. In an intention to treat analysis (ITT), all were censored at cLEE, last visit, death or Dec 31 2020. Incidence rates (IR) (events/1000 person-years) were calculated for each ARV overall and by ARV exposure (6-12 months, 1-2 years, and >2 years) to investigate cumulative Poisson regression was used to estimate the incidence rate ratio (IRR) of exposure (6-12 months, 1-2 years, and 2+ years) to investigate cumulative IRs were calculated for each ARV overall and by ARV (events/1000 person-years) were calculated for each ARV overall and by ARV.

Conclusion: This is the first large study systematically looking at contemporary ARVs and cLEE. cLEE is not uncommon and more frequent during the first year after initiating new ARVs. With 4 years median follow-up, we found no short term liver safety concerns with the use of INSTIs. Use of EFV and TDF are associated with an increased cLEE risk, while TAF and DRV are associated with lower risks, although TAF not statistically significant.

Adjusted IRR (95% confidence interval) of Chronic Liver Enzyme Elevation: ITT

624 COVID-19 OUTCOME IN PATIENTS WITH AND WITHOUT CIRRHOSIS
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Background: Severe outcomes of COVID-19 are associated with advancing age, and multiple medical comorbidities. The impact of COVID-19 on the clinical course of patients with cirrhosis has not been well studied. We determined the effect of SARS-CoV-2 infection on the hospitalization and survival rates of patients with cirrhosis.

Methods: Using ICD-10-CM codes, we identified all Veterans who were diagnosed with cirrhosis in the VA Corporate Data Warehouse and COVID-19 Shared Data Resource. Study cohort included Veterans who were tested for SARS-CoV-2 and had no history of organ transplantation or malignancies. Each SARS-CoV-2 positive case was propensity-score matched by demographics and comorbidities and had no history of organ transplantation or malignancies. Each SARS-CoV-2 positive case was propensity-score matched by demographics and comorbidities and had no history of organ transplantation or malignancies. Each SARS-CoV-2 positive case was propensity-score matched by demographics and comorbidities and had no history of organ transplantation or malignancies. Each SARS-CoV-2 positive case was propensity-score matched by demographics and comorbidities and had no history of organ transplantation or malignancies.

Results: Of 14,481 individuals included contributing 59,798 person years of follow-up, 1,427 experienced cLEE (95%CI: 23.8 [22.7, 24.1]). Overall at baseline, 32% were ART naive, 76% male, median (IQR) age was 47 (38.5), ALT was 23 (18, 30), CD4 was 541 (358, 744) cells, 63% were virally suppressed, and 15% +HCV, 4% +HBV. Median (IQR) follow-up time was 41(2.3, 3.5) years. There was no evidence of a cumulative ARV effect on cLEE incidence. cLEE was highest in the first 6-12 months post new ARV and declined thereafter; IR = 49.2 (44.3, 54.8) 6-12 months, 34.6 (31.5, 38.1) 1-2 years, and 23.9 (22.7, 25.1) 2+ years. Any use (vs. no prior use) of EFV and TDF were independently associated with an increased IRR of cLEE, and DRV was associated with a decreased risk of cLEE, Figure. INSTIs were not associated with cLEE. Figure. Conclusion: This is the first large study systematically looking at contemporary ARVs and cLEE. cLEE is not uncommon and more frequent during the first year after initiating new ARVs. With 4 years median follow-up, we found no short term liver safety concerns with the use of INSTIs. Use of EFV and TDF are associated with an increased cLEE risk, while TAF and DRV are associated with lower risks, although TAF not statistically significant.

Adjusted IRR (95% confidence interval) of Chronic Liver Enzyme Elevation: ITT
longer among SARS-CoV-2 positive individuals (7 vs 4 days, P < 0.0001). In Cox regression analysis, SARS-CoV-2 positivity was associated with a higher risk of all-cause mortality (HR 1.37, 95% CI 1.19-1.56).

**Conclusion:** Although patients with cirrhosis and COVID-19 were less often hospitalized, they had longer duration of hospitalization and were at higher risk of severe or critical illness and death.

**KAPLAN-MEIER CURVES TO DEMONSTRATE EVENT-FREE SURVIVAL AMONG SARS-CoV-2 POSITIVE AND NEGATIVE INDIVIDUALS.**

**Figure:** Kaplan-Meier curves demonstrate event-free survival among SARS-CoV-2 positive and negative individuals.

**ASSOCIATION OF HIV CONTROL AND IMMUNOSUPPRESSION WITH NADC RISK AMONG PATIENTS ON ART**

Brittney Dickey1, Elizabeth Yanik2, Zachary Thompson1, Greer Burkholder1, Mari Kitahata1, Richard Moore1, Jeffrey Jacobson2, W. Christopher Mathews7, Katerina Christopoulos1, Julia Fleming3, Chad Achenbach1, Anna E. Coghill1, Moffitt Cancer Center, Tampa, FL, USA, 2Washington University in St Louis, St. Louis, MO, USA, 3University of Alabama at Birmingham, Birmingham, AL, USA, 4University of Washington, Seattle, WA, USA, 5The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 6Case Western Reserve University, Cleveland, OH, USA, 7University of California San Diego, San Diego, CA, USA

**Background:** Since inception of highly active antiretroviral therapy in 1996, AIDS-related mortality has decreased, and life expectancy among people with HIV (PHIV) has increased. This has translated into an increased prevalence of age-related conditions including non-AIDS defining cancers (NADCs). Our study investigated the association of measures of immunosuppression and HIV control with NADCs with viral or non-viral etiology among PHIV on antiretroviral therapy (ART) in the US.

**Methods:** Patients who sought care at clinics within the CFAR Network of Integrated Clinical Systems (CNICS) between 1996-2016 were assessed for development of a primary NADC. Follow-up started at CNICS enrollment and ended at first NADC diagnosis, death, last CNICS visit, or last date of cancer verification (12/31/2016). To assess immune function and HIV control, we utilized three parameterizations of CD4 count and HIV-RNA viral load (VL): (1) CD4 or VL at ART initiation, (2) change in CD4 or VL following ART initiation; and (3) proportion of follow-up time at CD4 >500 cells/ul or VL < 50 copies/ml. To ascertain the association of these measures with risk of a viral NADC (anal, Hodgkin lymphoma, liver, oropharyngeal, penile, vulva, vaginal) or non-viral NADC (all other sites), Cox models adjusted for age, race/ethnicity, sex, year of ART start, prior HIV and HCV diagnoses (viral only), and tobacco (non-viral only) were used.

**Results:** Among 29,568 patients on ART, there were 410 non-viral NADCs and 213 viral NADCs. PHIV with a CD4 < 200 cells/ul at ART initiation had an 80% elevated risk for developing a viral NADC (aHR: 1.80; 95% CI: 1.12-2.87). Each increase of 100 cells/ul in CD4 after ART initiation decreased risk 14% (aHR: 0.86; 95% CI: 0.77-0.95), and 10% more follow-up time spent with a CD4 < 500 cells/ul was associated with decreased risk (aHR: 0.82; 95% CI: 0.78-0.86), even after accounting for CD4 at ART initiation. Risk of non-viral NADCs was also lower for those with 10% more time spent with CD4 > 500 (aHR: 0.89; 95% CI: 0.86-91). When examining HIV control only, 10% more time with VL < 50 copies/ml was significantly associated with decreased viral (aHR: 0.85; 95% CI: 0.82-0.89) and non-viral NADC risk (aHR: 0.88; 95% CI: 0.85-0.90).

**Conclusion:** This study demonstrates that even for PHIV on ART therapy, maintaining HIV control is associated with lower risk of both viral and non-viral NADCs.

**HIV-ASSOCIATED DIFFERENCES IN THE TUMOR IMMUNE MICROENVIRONMENT OF NADCs**

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1Moffitt Cancer Center, Tampa, FL, USA, 2Huntsman Cancer Institute, Salt Lake City, UT, USA

**Background:** Antiretroviral therapy has translated into increased life expectancy for people living with HIV (PWH), resulting in a growing number of patients surviving to older ages when non-AIDS-defining cancer (NADC) are more common. Our prior work has demonstrated that patients with HIV have higher cancer-specific mortality compared to patients without HIV. A novel approach to identify factors that drive poor cancer outcomes in PWH is needed; one compelling hypothesis is that HIV influences the molecular profile of cancers that develop in PWH.

**Methods:** Tissue microarrays (TMAs) from PWH diagnosed with either prostate or anal cancer were obtained from the AIDS Cancer Specimen Resource. Comparison TMAs were created from HIV-uninfected prostate and anal cancer patients selected from the biorepositories at Moffitt Cancer Center and Huntsman Cancer Institute. In addition, one TMA was created at Moffitt to include tumors from PWH diagnosed with a range of NADCs and matched tumors from HIV-uninfected patients diagnosed with the same cancer type. Slides were stained using the Akyra Biosciences’ OPAL™ 7-Color Automation IHC kit on the BOND RX autostainer (Leica Biosystems). After staining, slides were imaged using the Vectra®3 Automated Quantitative Pathology Imaging System, and multi-layer images were exported to the HALO Image Analysis Platform. We compared marker positivity by HIV status, adjusted for tumor site and patient age and race, using beta-binomial regression models.

**Results:** Multiplex immunofluorescence staining of tissues from 45 PWH (prostate cancer=22; anal cancer=14; other=9) and 238 HIV-uninfected patients (prostate cancer=216; anal cancer=5; other=17) demonstrated HIV-related differences in the tumor immune microenvironment. We stained for 15 different markers, and the abundance of six of these markers was significantly (P< 0.05) higher in tumors from PWH compared to tumors from patients without HIV, after adjustment for age, race, and tumor site. This included differences in infiltration of T-cells (CD8+ [OR=1.50] and delta-gamma [OR=1.81]), B-cells (CD20+ [OR=1.67]), and macrophages (CD163+ OR=1.98), as well as differences in expression of clinically targetable immune checkpoint molecules (PD-L1 [OR=3.83] and TIM3 [OR=1.76]). The regulatory cell phenotype (CD3+CD8+FOXP3+) was also statistically significantly more abundant in tumors from PWH (OR=2.19).

**Conclusion:** Our data indicate that NADCs developing in the setting of HIV are immunologically distinct.

**SPECIFIC EXOSOMAL PROTEOMIC PROFILE ASSOCIATED TO PLWH PRESENTING NADCs**

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1Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Madrid, Spain, 2Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain, 3Spanish National Cancer Research Center, Madrid, Spain, 4Hospital Universitario Rey Juan Carlos, Madrid, Spain, 5Hospital Universitario Infanta Elena, Valdemar, Spain, 6Universidad Complutense de Madrid, Madrid, Spain

**Background:** People living with HIV have an increased risk of developing different types of cancers (NADC). Non-AIDS Defining Cancers). However, it is poorly understood the underlying mechanisms that link up HIV infection with the development and progression of these cancers. Previous in vitro studies have suggested that exosomes derived from HIV infected cells can promote and enhance cancer. Herein, we have analyzed the proteomic profile of exosomes from HIV patients with active NADC to identify biomarkers associated to this condition.

**Methods:** Forty-five HIV patients were included: 15 on cART (HIV+ NADCs+), 15 on cART with NADC (HIV+ NADCs-), and 15 HIV-uninfected with NADCs (HIV- NADCs+). Fifteen healthy volunteers (HC) were included as reference. Size Exclusion Chromatography was employed to isolate plasma exosomes and Nanoparticle Tracking Analysis and microscopy-TEM to quantify and validate them. Proteins were extracted from exosomes, digested with trypsin and the resulting peptides were analyzed by liquid chromatography coupled with mass spectrometry. Proteins identification was carried out using the Mascot search engine through the Protein Discoverer software. Differential abundance of proteins between study groups was considered when the ratio of abundance was >2 or <0.5.
Results: Respect to HC, there were 74, 77 and 72 differentially expressed proteins in HIV-NADCs, HIV-NADCs- and NADCs/HIV- groups, respectively. Eighteen proteins were exclusive of NADC-HIV+ vs HC comparison, of which 11 were increased. Among these 11 proteins, 5 are related to cancer development and progression: hSODX, Proprotein convertase 9, Complement component C9, Beta ig-h3 and C4b-Lp-in contrast, one of the proteins diminished in NADC-HIV+ (GpX-3) is considered as anti-oncogenic factor, and curiously, its mRNA expression is downregulated by HIV-Tat. Moreover, there were 7 proteins exclusive of the comparison between HIV-NADCs- and HIV-NADCs+; among them two proteins associated to cancer development and metastasis were increased in NADC+HIV+: SAA1 and Thrombospondin 1. Interestingly, mRNA expression of SAA1 is upregulated by HIV-gp120.

Conclusion: Our results show the existence of a specific exosomal proteomic profile associated to concomitant HIV infection and NADCs, characterized by increased levels of proteins related to cancer promotion and decreased levels of anti-oncogenic factors. Of note, some of these proteins are considered targets regulated by HIV, supporting the contribution of the virus to the development and progression of cancers.

628 RISK FACTORS FOR 5-YEAR MORTALITY IN PEOPLE WITH HIV AFTER CANCER DIAGNOSIS

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Background: Racial disparities in cancer outcomes among people with HIV (PWH) have not been extensively explored in the context of HIV-specific clinical factors such as HIV viral load and immune status. We estimated 5-year survival and risk factors for 5-year mortality among PWH diagnosed with cancer in North America from 2000-2017.

Methods: We included PWH, ≥18 years old, participating in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of the IeDEA 1The Johns Hopkins University, Baltimore, MD, USA, 2University of Calgary, Calgary, AB, Canada, 3Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 4Kaiser Permanente Division of Research, Oakland, CA, USA, 5Stanford University, Palo Alto, CA, USA, 6University of Calgary, Alberta, BC, Canada, 7Yale University, West Haven, CT, USA, 8University of California San Diego, San Diego, CA, USA, 9National Cancer Institute, Rockville, MD, USA, 10University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Results: There were 4556 cancer diagnoses (827 ADC, 2832 NADC, 897 VAC) among 4103 patients contributing 12185 person-years. Five-year survival was 50.1% (46.2%, 53.8%) for ADCs, 38.5% (36.4, 40.6%) for NADCs, and 32.9% (28.9, 37.0%) for VACs. With age as the timescale, we estimated 5-year survival by cancer type using Cox proportional hazards models including race/ethnicity, viral suppression, CD4 count, AIDS-defining illness (ADI) prior to cancer, and calendar year of cancer diagnosis.

Conclusion: Among patients with anal cancer, lung cancer, or NHL living with HIV, a low CD4 count at the time of diagnosis was associated with an increased risk of individuals subsequently dying from their cancer. These results are preliminary, and additional analyses need to be performed that further adjust for cancer stage at diagnosis and cancer treatment. Nonetheless, our findings support that immunity plays a crucial role in the control of certain malignancies and preventing relapse and death. Optimizing antiretroviral therapy to suppress HIV replication and restore immune function should be an important component of cancer treatment.
KSHV-infected B cell plasmablasts. Inhibition of STAT3 can induce apoptosis in PEL cells (Aoki Y. et al., Blood, 2003 101:1535), and signaling by KSHV-encoded viral IL-6 through STAT3 is believed to be important in KSHV-MCD pathogenesis. We explored the potential of JAK inhibitors for use in PEL and KSHV-MCD.

Methods: JAK Inhibitors (ruxolitinib, AZD1480, baricitinib, peficitinib, and pacritinib) and other kinase inhibitors were purchased from commercial sources; pacritinib was also provided by CTI BioPharma. PEL cell lines (JSC-1 and BCBL-1) were treated with the inhibitors, and their viability assessed using the CellTiter-Glo® Luminous Cell Viability Assay and flow cytometry. To assess effects on cellular gene expression, mRNA sequencing was applied, and key results were verified using real-time (RT) PCR.

Results: As shown by flow cytometry and trypan blue counting, 1 µM pacritinib efficiently inhibited cell growth and induced apoptosis of PEL cell lines and was superior to the other JAK inhibitors tested. In addition to JAK2, pacritinib targets FLT3 and IRAK1; the possibility that these might contribute to the effect in PEL using small molecule inhibitors was explored. Several FLT3 inhibitors also inhibited growth of PEL cells in vitro, suggesting that FLT3, which is involved in B cell development, may play a role in pacritinib’s growth inhibition of PEL. mRNA sequencing and RT-PCR showed that several key host genes including several cytokines and IL-6 were downregulated by pacritinib. Finally, pacritinib suppressed KSHV viral-IL6(vIL-6)-induced IL-6 production by peripheral blood mononuclear cells, which may model an important step the pathogenesis of KSHV-MCD.

Conclusion: Pacritinib inhibited cell growth in PEL lines. It also downregulated a number of cellular genes believed to be important for PEL growth and for KSHV-MCD pathogenesis. These effects may stem from its simultaneous inhibition of multiple kinases including Jak2, IRAK1, and FLT3. These results suggest that pacritinib warrants testing for the treatment of KSHV-MCD and PEL.

631 IFNγ-IL18 AXIS CYTOKINES DISCERN MYCOBACTERIAL AND KSHV INFLAMMATORY SYNDROMES IN PWH

Joseph Rocco1, Ramya Ramaswami2, Kathryn Lurain1, Elizabeth Laidlaw1, Andrea Lisca1, Frances Galindo1, Adam Rupert1, Denise Whirty1, Maura Manion1, Robert Yarchoan1, Irini Sereti1

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Background: Mycobacterial immune reconstitution inflammatory syndrome (myco-IRS) occurs in people with HIV (PWH) who are also at risk of Kaposi sarcoma herpesvirus (KSHV)-associated inflammatory diseases (KAD) that are associated with elevated IL-6 and clinically resemble myco-IRS. Differentiating these syndromes is essential as treatments vary markedly. IFNγ-IL18 axis biomarkers can distinguish pediatric hyperinflammatory syndromes and have been implicated in both myco-IRS and KAD pathogenesis. We evaluated clinical and immune biomarkers in patients with myco-IRS or KAD to identify markers that could differentiate these syndromes.

Methods: Plasma samples were obtained from PWH on antiretroviral therapy who were treated with the inhibitors, and their viability assessed using the CellTiter-Glo® Luminous Cell Viability Assay and flow cytometry. To assess effects on cellular gene expression, mRNA sequencing and RT-PCR were performed. IFNγ and IL18 were measured by ELISA. Significance was assessed using Wilcoxon test, principal component (PCA), and receiver operating characteristic (ROC) analysis.

Results: Overall, 27 PWH were included (median age 39yrs [IQR 33-45]) with 59 (77%) cis-men. Fifty had myco-IRS (tuberculosis, n=27; non-tuberculous, n=29), and 27 had KAD. There was no difference in CRP, IL6, ferritin, CD4 T-cells, or HIV viral load between the two groups. PCA incorporating all biomarkers revealed distinct clustering of the two inflammatory syndromes (Fig1A). The 2 patients with myco-IRS and possible KAD mapped to separate inflammatory clusters (stars) suggesting unique pathophysiology in each. There were higher IFNγ and CXCL9 levels (T-cell activation) in myco-IRS (IFNγ 135pg/mL [IQR 35.8-333]; CXCL9 786pg/mL [IQR 429-1863]) vs KADs (IFNγ 25.4pg/mL [IQR 19.4-37.6]; CXCL9 2722pg/mL [IQR 171-437]) (p<0.001). IL18 (inflammascalmon activation) was increased in KAD (IL18 4202pg/mL [IQR 2042-5923]) compared to myco-IRS (IL18 1018pg/mL [IQR 670-1705]) (p<0.001). The IL18/CXCL9 ratio showed the greatest discrimination capacity for KAD (Fig1B).

Conclusion: Although clinically indistinguishable, myco-IRS and inflammatory KAD demonstrate unique immune profiles involving the IFNγ-IL18 axis suggesting different pathogenesis. These biomarkers, after validation, may help risk stratification and diagnosis of patients even in resource-limited settings, but also highlight possible therapeutic targets of these distinct hyperinflammatory syndromes.

632 STUDY OF CHARACTERISTICS AND OUTCOMES OF KSHV INFECTED CYTOKINE SYNDROME (KICS)

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Background: KSHV inflammatory cytokine syndrome (KICS), caused by Kaposi sarcoma herpesvirus (KSHV), is characterized by severe inflammatory signs (elevated C-reactive protein, elevated KSHV viral load) and symptoms without evidence of multicentric Castleman disease (MCD). KICS, predominantly occurs among people with HIV (PWH) and Kaposi sarcoma (KS), and has overlapping clinical features of MCD and primary effusion lymphoma (PEL). We present findings from the largest prospective study of KICS.

Methods: The primary objective was to evaluate KICS natural history. There were broad eligibility criteria to allow thorough diagnostic evaluation for KICS and exclusion of other inflammatory disorders. Participants (pts) with confirmed KICS (without PEL, MCD or other causes of inflammation) were assigned to either an observation arm to treat KS with standard therapy or to a KICS treatment arm to receive rituximab-based therapies, high-dose dexamethasone and vincristine (AVZT/GVC) or other rational therapies, such as tocilizumab. Results: Seventy-six pts were enrolled from 2011 to 2022. Twenty-eight pts did not meet further KICS criteria, as inflammatory symptoms were associated with PEL (20 pts), MCD (2 pts), HIV infection alone (5 pts), or paraganglioma (1 pt). Therefore, following initial evaluation, 48 pts (47 PWH) had confirmed KICS, and all but 1 had concurrent KS. In 47 pts with confirmed KICS and HIV infection, 43 pts (91%) were on antiretroviral therapy (IQR: 23-4631), and median CD4+ T cell count was 86 cells/µL (IQR: 32-155). Among all pts with confirmed KICS, 27 pts (56%) received KS-focused therapy alone, 18 pts (35%) received rituximab-based therapy [with liposomal doxorubicin (15 pts) or paclitaxel (2 pts) or without chemotherapy (1 pt)], 1 pt received AVZT/GVC, 1 pt received tocilizumab. Ten of 18 pts (55%) who received rituximab-based therapies had clinical benefit with improvement of KICS signs and symptoms within 8 weeks of treatment. For pts with confirmed KICS, the median overall survival was 45.8 months (95% confidence interval: 20.3 – not reached) (Figure 1).

Conclusion: KICS is a distinct diagnosis of exclusion that often occurs with KS and carries a relatively poor survival. Pts should have MCD and PEL ruled out prior to KICS diagnosis. Rituximab-based therapies may have a role in treatment of a subset of pts with KICS.
633 KAPOSI SARCOMA IN ART-TREATED PLWH: DISTINCT VIRAL AND IMMUNE CHARACTERISTICS

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1Research Institute of McGill University Health Centre, Montreal, QC, Canada, 2Sorbonne Université, Paris, France, 3Research Institute of McGill University Health Centre, Montreal, France, 4McGill University Health Centre, Montreal, QC, Canada

Background: Recent reports describe the reemergence of HHV-8-induced Kaposi sarcoma (KS) in people living with HIV (PLWH) despite efficient antiretroviral therapy (ART). We aimed to assess the influence of immunological and virological factors in the development of KS in ART-treated PLWH compared to HIV-uninfected people with classic KS.

Methods: 4 groups of 11 participants were compared: 1. ART-treated PLWH with KS (KS HIV+), 2. Age-matched ART-treated PLWH without KS (HIV+), 3. HIV-uninfected patients with classic KS (KS HIV-), 4. Age-matched HIV-uninfected people without KS. We assessed CD4/CD8 ratios, circulating cytokines by ELISA multiplex, anti-HHV-8 IgG levels with an in-house cell-based assay, anti-HHV-8 specific T-cells by ELISPOT, and circulating and skin T-cells phenotypes by flow cytometry. HHV-8 viral loads (VL) were quantified by digital-droplet PCR and next-generation sequencing was performed.

Results: All KS participants presented with limited skin disease. KS HIV+ were younger than KS HIV- (53 vs 75yo, p<0.01). CD4/CD8 ratios were lower in KS HIV+ compared to KS HIV- (p=0.01) and to HIV+ (p<0.05). In KS HIV+, anti-HHV-8 IgG levels were higher compared to KS HIV- (p=0.02, Figure 1) and frequency of specific T-cells was low but similar. Circulating and tissular CD4 T-cells of both KS HIV+ and KS HIV- expressed a similarly high frequency of senescence markers (CD57+/CD28-) and PD1, higher than their own control group. Among cytokines, IL-10 levels were higher only in KS HIV- (p=0.02). HHV-8 VL were lower in KS HIV+ than in KS HIV- in plasma (p=0.02) and PBMCs (p=0.04), but were similar in skin biopsies. HHV-8 genetic subtypes A and C were similarly isolated in both KS groups, and a newly identified variant was found in two KS HIV- Inuit patients from Northern Canada.

Conclusion: Compared to age-matched PLWH, ART-treated PLWH with KS exhibited features of early immune senescence, with a lower CD4/CD8 ratio and increased frequency of CD57+/CD28- T-cells. Despite the younger age, senescent T-cells frequency was similar among KS HIV+ and KS HIV-. However, anti-HHV8 IgG levels were higher in KS HIV+ compared to KS HIV-, which was associated with lower circulating HHV-8 DNA and IL-10 levels. Although HHV-8 strains did not significantly differ between groups, we could isolate a new HHV-8 variant. Altogether, early immune senescence/exhaustion seems involved in the development of KS in ART-treated PLWH. Such insights will help developing therapeutic strategies to reduce KS-induced stigma.

634 IMPLICATIONS OF CERVICAL CANCER SCREENING AND TREATMENT PROGRAM ROLL OUT IN UGANDA

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Background: At 54.8 cases per 100,000 women, Uganda is one of the highest cervical cancer (CxCa) burdened countries in the world. Women living with HIV (WLHIV) are 6 times greater risk of developing CxCa, compared to women without HIV. To reduce the occurrence of CxCa among WLHIV, the Uganda Ministry of Health (MOH), with support from the US President’s Emergency Plan for AIDS Relief (PEPFAR) through the PEPFAR agencies (CDC, USAID, DOD) and Implementing Partners, began a CxCa screening and treatment of pre-cancerous lesions program in October 2020. Here, we describe initial program performance and implications for national scale-up.

Methods: We supported the design, development and printing of standards, guidelines and monitoring and evaluation tools for the program; we also trained national, regional and health facility managers to support the roll-out of the program. MOH procured and distributed commodities required for CxCa screening and treatment of pre-cancerous lesions. To assess performance, we performed a retrospective analysis of CxCa screening and care cascade data from October 2020 through March 2022. We extracted data from the PEPFAR DATIM reporting system. We calculated the proportions of eligible WLHIV on antiretroviral therapy (ART) who were screened, those who screened positive (positivity rate), and those who received treatment for pre-cancerous lesions among those screened positive (treatment rate).

Results: Overall, 543,639 WLHIV on ART aged 25–49 years were eligible for screening during October 2020–October 2022. By March 2022, 47% (n=255,451) of eligible WLHIV were screened, with a positivity rate of 6% (n=14,378) and treatment rate of 64% (n=9,329). Percentage of WLHIV screened increased over time, with 27% (146,033/543,639) screened during October 2021-March 2022 compared to 20% (109,418/543,639) screened in a whole year period (October 2020-September 2021). Treatment rates increased over time, with 75% (5,695/7,623) treated during October 2021—March 2022 compared to 54% (3,634/6,755) during October 2020—September 2021.

Conclusion: Improvements in proportion screened and treatment rate were due to consistent commodity distribution, enhanced technical support, and implementation of quality improvement innovations. Additional efforts are needed to ensure all WLHIV who are screened positive receive treatment, including barriers to timely referrals.

635 INTEGRATING POINT-OF-CARE HPV TESTING INTO HIV CARE FOR KENYAN WOMEN LIVING WITH HIV

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Background: Current cervical cancer screening methods for women living with HIV (WLHIV) in Kenya are underutilized, including visual inspection of the cervix with acetic acid (VIA) and/or of Lugol’s iodine (VILI). Identifying WLHIV at the
highest risk for cervical cancer could motivate VIA/VILI screening and improve uptake. We integrated Xpert HPV testing for high-risk human papillomavirus (HR-HPV) within routine HIV care with the aim of increasing the overall uptake of cervical cancer screening among WLHIV enrolled in HIV care at Kenya’s national referral hospital.

**Methods:** WLHIV aged ≥18 years enrolled in HIV care at Kenyatta National Hospital (KNH) HIV clinics were eligible and approached for participation during their routine HIV clinic visits between September 2017 and February 2022. We extracted medical records among consenting WLHIV to establish baseline VIA/VILI uptake. Participants were offered Xpert HPV testing if they had not previously received VIA in the last 12 months. Cervical smears were collected among consenting WLHIV by study nurses and analyzed on the Gene Xpert platform in the HIV care clinic molecular laboratory. Results were provided during that same HIV care visit and women with HR-HPV were referred for VIA/VILI in the HIV clinic.

**Results:** Overall, 691 WLHIV were enrolled. The median age was 42 years (IQR 37–48) and 72% of participants had secondary education or above. Forty-six percent of participants were married, 63% had a regular source of income, and 47% had a partner known to be living with HIV. Only 25% of participants were previously screened for cervical cancer. Among these not previously screened (n=518), most (95%) accepted Xpert HPV. Prevalence of HR-HPV was 35% (232/656), 10% HR-HPV-16, 8% HR-HPV-18/31 and 82% for other 11 HR-HPVs not individually genotyped by Xpert HPV. The median time to return Xpert HPV results was 60 minutes (IQR 60–80). All the results were available in the same HIV clinic visit. Overall, 96% of WLHIV with positive Xpert HPV results subsequently accepted and received VIA/VILI assessment; of those, 26% had results predicting cervical abnormalities.

**Conclusion:** In this study among Kenyan WLHIV, integrating Xpert HPV into HIV care was feasible with high uptake and prevalence of HR-HPV. WLHIV with HR-HPV almost universally completed referrals for VIA/VILI which frequently detected cervical abnormalities. Xpert HPV could potentially enhance cervical cancer screening programs for WLHIV in high-burden settings.

**636 PROGRESS TOWARDS THE ELIMINATION OF CERVICAL CANCER AMONG WOMEN LIVING WITH HIV**

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**Background:** Acquisition and persistence of carcinogenic human papillomavirus (HPV) infections and the incidence of precancerous lesions and invasive cervical cancers are all increased for women living with HIV (WLHIV) compared with their HIV-negative peers. To reduce new cervical cancer cases among WLHIV, the U.S. President’s Emergency Plan for AIDS Relief (PEPFAR) supports regular cervical cancer screening, precancerous lesion treatment, and referral for treatment of invasive cervical cancers for women accessing routine HIV services. We describe cervical cancer screening and treatment results among WLHIV in PEPFAR-supported programs.

**Methods:** PEPFAR programs in 14 countries reported data semiannually at the end of March (Quarter 2) and September (Q4), 2018 to 2022. We report the absolute and relative number of WLHIV screened for cervical cancer for their first lifetime screen, routine follow up after prior negative screens, and after precancerous lesion treatment. Screen results are reported as negative, positive for precancerous lesions, and positive for suspected invasive cervical cancer. We describe the number and proportion of precancers treated with ablative and excisional therapies.

**Results:** Between April 2018 and March 2022, 4.5 million cervical cancer screenings were completed; 3.7 million (82.4%) were first time screens. Screen positivity for precancerous lesions was 5.1% (229,208 positive screens), and 0.9% (40,152 women) were referred to a higher level of care for suspected cancers. A total of 169,017 precancerous lesions were treated; 19.7% (33,289) with excisional, and the remainder with ablative therapies. The proportion of ablative treatments done with cryotherapy declined by 21.0%, while the proportion using thermal ablation increased by 26.1%, when comparing 2022 to 2018 semiannual rates. The precancerous lesion treatment rate increased from 58.1% in 2018 to 83.1% in 2022.

**Conclusion:** Offering cervical cancer screening services within ART clinics reached 4.5 million WLHIV at risk for cervical cancer—a significant contribution to the ongoing global effort to eliminate cervical cancer. PEPFAR programs should continue to optimize cervical cancer treatment modalities best suited to each location and to further increase the rising precancerous lesion treatment rates, to ensure that women have access to excellent cervical cancer screening and appropriate precancer treatments.
in our Ryan White HIV/AIDS Program (RWHAP) clinic evaluating engagement and retention in anal cancer screening services identified a significant gap in engagement in care among those eligible. This highlighted a need to identify and address barriers to engagement in screening services.

**Methods:** Semi-structured qualitative interviews were performed on people who were receiving care in the high resolution anoscopy (HRA) clinic at the University of Virginia. Participants were at least 35 years old, receiving HIV care in the clinic and had at least one HRA performed in the clinic. Recruitment was carried out by convenience sampling via internal clinic advertising and a tailored mobile app. Interviews were constructed using the health belief model and themes explored included perceived risks, benefits, and barriers to screening, as well as self-efficacy and cues to action.

**Results:** Prominent barriers to engaging in high resolution anoscopy (HRA) services included pain, prior sexual trauma, prolonged recovery after biopsy, and fear of new cancer diagnosis. Notable motivators to engaging in HRA services were increased perceived risk of cancer, sedation with anxiolytics, desire to maintain health as well as trust in RWHAP clinic providers and staff.

**Conclusion:** Our findings highlight the importance of eliciting patient perspectives as a powerful tool when evaluating and improving on a screening program’s performance. There is a desire to meet the needs of those receiving HRAs and adapt practices when it comes to those who have experienced prior sexual trauma. There is also a need to promote awareness of anal cancer screening services to PWI and provide anticipatory guidance when counseling people on the HRA procedure.

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### 642 RISK FACTORS FOR ANAL DYSPLASIA AND LINKAGE TO HRA IN TRANSGENDER WOMEN

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2. National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
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5. HIPS, Washington, DC, USA
6. University of Maryland Baltimore County, Baltimore, MD, USA

**Background:** Studies estimate that transgender women (TGW) have a high prevalence of high-risk HPV (HR-HPV) and anal intraepithelial lesions (1), and early intervention improves outcomes (2). We examined risk factors associated with anal dysplasia (AD) and linkage to high resolution anoscopy (HRA) in a sample of TGW with and without HIV.

**Methods:** We recruited a convenience sample of TGW in DC from 4/2021–9/2022. Data collected included: demographics; serum samples; anal swabs for cytology and HPV genotyping. We defined AD as a cytology diagnosis of atypical squamous cells of undetermined significance, low-grade or high-grade intraepithelial lesions (HSIL). Current use of gender affirming hormones (GAH) was defined as self-report of use, or serum estradiol level higher than 60 pg/mL (estrogen) and a testosterone level below 264 ng/dL (androgen blocker).

Participants with AD were scheduled for off-site HRA. We used chi-square tests to compare differences between AD risk factors.

**Results:** Of 62 TGW with adequate anal cytology samples, most were black (83%), stably housed (55%), engaged in anal receptive sex within 12 months (77%), and on GAH (56%). Only 12 (19%) recalled receiving an HPV vaccine. Of 43 (69%) patients with HIV, 15 (35%) had HIV viral load >200 copies/mL, median CD4 count was 619, and 22 (47%) had previous anal cancer screening. AD was found in 29 (47%), while 45 (74%) tested positive for HR-HPV. AD was associated with the presence of HR-HPV (p=0.04), and with black race (p=0.03), but was not significantly associated with current GAH use or HIV status (Table 1). In TGW with HIV, HIV viremia was not associated with a higher risk of AD: 10 (44%) AD with viremia and 13 (56%) AD in those without (p=0.2).

Of all TGW with AD, 23 (79%) had HRA scheduled, but only 6 (26%) attended, with HSIL found in 2 patients. A year after initial screening, 16 TGW had repeat anal samples collected. On repeat, 4 (25%) cleared anal HR-HPV, and 4 (25%) no longer had AD, including one with known HSIL on HRA.

**Conclusion:** Our findings highlight the high prevalence of HR-HPV and AD in TGW regardless of HIV status, HIV suppression, age or use of GAH. In this high-risk population, we found low rates of prior HPV vaccination, and limited HRA attendance despite facilitated linkage. Future studies should identify longitudinal risk factors for persistence of HR-HPV or AD, and strategies for enhancing HPV vaccination, anal cancer screening, and linkage to HRA in TGW.
**Table 1: Association of risk factors with abnormal anal cytology.**

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**Methods:** A cross-sectional study of 54 anal biopsies obtained from 47 MSM living with HIV who participated in an anal screening program was performed. In these samples, we assessed multiple lymphocyte and myeloid immunological subsets by flow cytometry, in addition to histological examination. Selected potential biomarkers were further assessed by immunohistochemistry.

**Results:** Resident Memory T cells expressing CD103 were less frequent in pathological biopsies (Low/high-grade-SIL (LSIL/HSIL)), with a more pronounced effect on the CD4+ T cell subset (p=0.024). Increases in the frequency of Natural Killer cells (NK) expressing CD16 (p=0.030) and overall NK activation measured by HLA-DR (p=0.018) were also associated with pathological samples. Furthermore, potentially immune suppressive subsets, including CD15+CD26+ neutrophils, gradually increased as the anal lesion progressed (p=0.012). Staining of CD15 by immunohistochemistry confirmed the association between the presence of this biomarker in the epithelium and SIL, with a sensitivity of 80% and specificity of 71% (AUC 0.762) for the correlation with HSIL.

**Conclusion:** Immunological tissue analyses revealed a complex immunological environment where the balance between resident effectors and immune suppressive subsets was tilted towards the second in pathological samples. Neutrophil infiltration determined by CD15 staining, may represent a valuable biomarker associated to the grade of dysplasia.

**NADIR CD4 AND ANAL CYTOMETRY PREDICT DEVELOPMENT OF INVASIVE ANAL CANCER**

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**Background:** The long-term natural history of anal high-grade squamous intraepithelial lesions (HSILs) is incompletely characterized. We aimed to evaluate the effects of readily available patient characteristics on the risk of developing IAC in a longitudinal cohort of people with HIV (PWH).

**Methods:** Retrospective inception cohort analysis of PWH between 2006-2021. Follow-up ended on 31 Dec 2021, whichever occurred first.

**Results:** The cohort included 3,967 PWH followed for a median of 5.5 years (up to 13.8 years), and 26 developed incident IAC during follow-up. PWH had a median of 2 anal cytologies. Patient characteristics included: median age 34, 10% females, 36% non-white, 78% men who had sex with men and 33% smokers. Most were on ART (91%). Those with VL < 400 copies/ml at the beginning and end of follow-up were 68% and 89%, respectively. PWH had a median nadir CD4 count of 267 cells/mm3 (IQR: 102, 454) and 39% had a CD4 count nadir < 200. At the first cytology test, 12% had HSIL, while cumulatively 23% developed HSIL during follow-up. In weighted Cox models, independent predictors of developing IAC were being ever HSIL (HR:3.92, 95% CI:1.76-8.75, P=0.001) and nadir CD4 < 200 (HR:4.56, 95% CI:1.54-12.845, p=0.004).

Neither age, sex, race, smoking status, VL or HIV risk factor predicted the IAC development, Harrell’s C = 0.77. Comparing those with HSIL to those with less than HSIL, 5-year predicted probabilities of IAC were 1.67% and 0.22%, respectively. Comparing those with nadir CD4 < 200 to those ≥200, 5-year IAC probabilities were 1.23% and 0.15% (Figure).

**Conclusion:** In this 15-year longitudinal cohort, HSIL and nadir CD4 count were independent predictors of IAC. Unbiased estimates of the natural history of IAC observed in inception cohorts should inform shared decision-making discussions regarding anal cancer screening.

**TISSUE-RESIDENT T CELL RESPONSES IN HIV+ PATIENTS WITH HPV-DRIVEN ANAL DYSPLASIA**

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**Background:** Men who have sex with men (MSM) and people living with HIV (PLHIV) have up to 150x increased risk of developing human papillomavirus (HPV)-driven anal cancer compared to the general population. Despite receiving antiretroviral therapy (ART), PLHIV can have poor HPV clearance resulting in high-grade squamous intraepithelial lesions (HSIL), an anal cancer precursor. High CD103+ CD8+ tissue-resident memory T cell (CD8+ TRM) numbers are associated with improved survival in HPV+ oropharyngeal and cervical cancer. Their role in anal cancer has not been investigated. We aim to delineate mucosal immunity responses in MSM (including PLHIV) that result in HPV clearance.

**Methods:** Anal biopsies from 67 MSM in the Study of the Prevention of Anal Cancer were studied. Multiplexed microscopy was used to determine the effects of HIV co-infection with high-risk HPV16 on local T cell profile. Mann-Whitney t-tests and Spearman correlation were performed. Whole transcriptomic differences between the dysplastic lesion (DL) and peri-lesion (PL) areas were assessed in HSIL regression (R; n=2) or non-regressive disease (NR; n=2) (NanoString GeoMx Digital Spatial Profiler). gProfiler identified key pathways and cell populations.

**Results:** Higher numbers of total CD4+ T cells were evident in MSM with HIV compared to those without (Fig. 1A). While total CD8+ T cells were similar in both cohorts, CD8+ TRM were lower in MSM with HIV (Fig. 1B-C). In HPV+ HSIL, ‘classical’ CD103+ CD4+ T cells and CD4+ TRM numbers were lower in MSM with HIV compared to those without (Fig. 1 D-E). In both cohorts, total CD8+ T cell counts positively correlated with CD4+ T cells (HIV+: r=0.504, p<0.01; HIV-: r=0.617, p<0.001) and CD8+ TRM (HIV+: r=0.727, p<0.0001; HIV-: r=0.747, p<0.0001). Only in MSM with HIV did CD4+ T cell numbers positively correlate with CD8+ TRM numbers (r=0.450, p=0.0509). In PL, 144 (NR) and 218 (R) genes, and in DL 140 (NR) and 73 (R) genes, were upregulated. Investigation of canonical gene sets found immune response and regulation to tumour cell signals in the R cohort compared to MHC regulation and viral gene expression signals in NR.
Conclusion: HIV infection is characterized by low CD8+ T cell and classical CD4+ T cell numbers. Low CD8+ T cell numbers may be associated with CD4+ T cell tissue lymphopenia resulting in poor HPV clearance. Even in the ART era, the immune response signals in R but not NR suggest differential cellular and molecular signals in response to HPV infection.

646 CORONARY ARTERY PLAQUE COMPOSITION AND SEVERITY RELATE TO THE INFLAMMASOME IN HIV

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Background: The inflammasome, an innate immune system component that regulates the secretion of powerful proinflammatory cytokines, has increased activity in people with HIV (PWH) despite antiretroviral therapy (ART). However, the relationship between the inflammasome and coronary artery plaque composition and severity is unknown.

Methods: The Randomized Trial to Prevent Vascular Events in HIV (REPREVE) enrolled PWH aged 40-75 on stable ART with low-moderate ASCVD risk. In a subset of participants, cross-sectional relationships between inflammasome activation markers and coronary plaque indices by coronary computed tomography angiography were explored. The relationships of caspase-1, IL-1β, and IL-18 to five qualitative measures of plaque burden and composition were assessed via binary (Leaman score) or ordinal logistic regression, adjusted for age, natal sex, LDL, hypertension, smoking, current and nadir CD4, and ART duration. Ordinal models assumed proportional adjacent category odds.

Results: Among 752 participants, median age was 50, 18% were women, and median ASCVD risk score was 4.5%. Median current CD4 was 601, 51% had a nadir CD4 < 200, and 98% had a HIV viral load < 400. Caspase-1 and IL-18 were consistently associated with multiple measures of inflammasome activation markers were seen in men and those with persistent viremia. Higher IL-18 was associated with 5% greater odds of having a Leaman score >5 (vs ≤5), but this did not reach statistical significance (P=0.08).

Conclusion: Among treated PWH at low-to-moderate ASCVD risk, higher inflammasome activation markers were seen in men and those with persistent viremia. Higher IL-18 was consistently associated with multiple measures of calcified and non-calcified plaque as well as Leaman score, which integrates degree of stenosis and plaque location and composition into a single index. Given this and Leaman score’s (≥5) known association with increased major adverse cardiovascular events (MACE) in the general population, future research is needed to determine whether anti-inflammasome therapeutics may mitigate the progression of atherosclerotic plaque or MACE in PWH.

Table: Adjusted Odds Ratios per 25% Higher Caspase-1, IL-1β, and IL-18

<table>
<thead>
<tr>
<th>Measure</th>
<th>Caspase-1</th>
<th>IL-1β</th>
<th>IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest vs lowest tertile</td>
<td>1.05 (0.87, 1.27)</td>
<td>1.05 (0.87, 1.27)</td>
<td>1.05 (0.87, 1.27)</td>
</tr>
<tr>
<td>Non-calcified plaque segments</td>
<td>0.98 (0.95, 1.02)</td>
<td>0.98 (0.95, 1.02)</td>
<td>0.98 (0.95, 1.02)</td>
</tr>
<tr>
<td>Calcified plaque segments</td>
<td>1.01 (0.95, 1.07)</td>
<td>1.01 (0.95, 1.07)</td>
<td>1.01 (0.95, 1.07)</td>
</tr>
<tr>
<td>Total plaque segments</td>
<td>1.01 (0.95, 1.07)</td>
<td>1.01 (0.95, 1.07)</td>
<td>1.01 (0.95, 1.07)</td>
</tr>
<tr>
<td>Mixed stenosis</td>
<td>1.01 (0.95, 1.06)</td>
<td>1.01 (0.95, 1.06)</td>
<td>1.01 (0.95, 1.06)</td>
</tr>
<tr>
<td>Leaman score</td>
<td>1.04 (0.97, 1.12)</td>
<td>1.04 (0.97, 1.12)</td>
<td>1.04 (0.97, 1.12)</td>
</tr>
</tbody>
</table>

* Higher vs equal or lower tertile.

647 BURDEN OF CORONARY DISEASE IN TRANSGENDER WOMEN WITH AND WITHOUT HIV

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Background: Cardiovascular disease (CVD) burden in transgender women (TW) may be affected by gender-affirming hormonal therapies (GAHT), HIV and antiretroviral therapy (ART), but few data exist comparing TW on contemporary GAHT and ART to well-matched controls. We compared CVD burden and sex hormone profiles between TW and matched cisgender men (CM).

Methods: Adult TW on GAHT (n=31) were recruited from Houston, TX and Baltimore, MD for a cross-sectional study (2018-2020). CM (n=60) from the Multicenter AIDS Cohort Study were matched (2:1) to TW on HIV serostatus, age ±5 years, race/ethnicity, BMI category and ART type. All HIV+ persons had HIV-1 RNA < 50 copies/ml on ART. Subclinical coronary atherosclerosis was measured by computed tomography (CT) angiography. Serum was collected concurrent to CTs; sex hormone concentrations were measured centrally. Student’s t, Kruskal-Wallis or Chi-square tests compared groups; correlations were determined using Pearson or Spearman testing.

Results: Overall, median age was 53 years and BMI 29 kg/m²; 73% were non-white and 75% HIV+. Only 3% of TW had testosterone suppression (< 50 ng/dl, TW-S). Traditional CVD risk factors were similar, except that the subset of TW-S had higher BMI (24 kg/m²) than TW with detectable testosterone (TW-T) and CM. Compared to CM and TW-T, TW-S with and without HIV had similar prevalences of calcified and mixed plaque, but none had non-calcified plaque or stenosis >50% (Table). Estradiol but not T correlated with mixed plaque (r=0.27, p<0.01) and total plaque and total stenosis (both r=-0.21, p=0.05).

Conclusion: In older TW with suppressed total testosterone on GAHT, no non-calcified coronary plaque or advanced stenosis was observed, while CM and TW with detectable testosterone had equivalent subclinical CVD burden. Observations occurred independent of HIV serostatus and despite similar traditional CVD risk factor profiles to CM and more obesity among TW with suppressed testosterone. Longitudinal studies to understand relationships between GAHT and CVD risk in TW are needed.

Table: Median hormone profiles between TW and CM

<table>
<thead>
<tr>
<th>Hormone</th>
<th>CM (n=31)</th>
<th>TW-T (n=30)</th>
<th>TW-S (n=31)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dl)</td>
<td>5 (3, 9)</td>
<td>5 (3, 9)</td>
<td>&lt; 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>262 (24, 765)</td>
<td>262 (24, 765)</td>
<td>0.15 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free testosterone (pg/ml)</td>
<td>126 (6, 246)</td>
<td>126 (6, 246)</td>
<td>0.15 (7.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
| Frequency of median and interquartile range presented *p<0.05 between CM and TW

CVD risk factors: TW+transgender women with detectable total testosterone (≥50 ng/dl), TW-S+transgender women with undetectable total testosterone.
Background: HIV infection is associated with increased immune activation and inflammation, higher coronary artery disease (CAD) prevalence, and adverse cardiovascular outcomes. Here, we relate pericoronary adipose tissue (PCAT) density, an index of pericoronary inflammation, to the presence and extent of CAD in people with HIV (PWH) and non-HIV controls.

Methods: In this baseline analysis of the Mechanistic Substudy of the Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) and non-HIV controls from the Framingham Heart Study (FHS), we related CT-derived PCAT density (Fig. 1A) to presence and extent (Leaman score) of CAD. Coronary artery calcium (CAC) score, and vulnerable plaque (VP) features in PWH using multivariable logistic regression analysis. In addition, we compared the PCAT density between PWH in REPRIEVE (n=727) and age, sex, body mass index (BMI), and CAC score–matched non-HIV controls (n=590) from the FHS by t test and random effects modeling, further adjusting for additional clinical covariates.

Results: The REPRIEVE analytic cohort included 608 males and 119 females (median age 51 years). In REPRIEVE, PCAT density was higher in those with coronary plaque, CAC score >0, vulnerable plaque, and high CAD burden (Leaman >5) (p < 0.001 for all, Fig. 1B). In a multivariable analysis, PCAT density was related to prevalent CAD on coronary CTA (aOR per 10 HU) = 1.51; 95% CI: 1.26–1.80; p < 0.01), adjusted for clinical cardiovascular risk factors, BMI, systemic immune/inflammatory biomarkers, and HIV-specific parameters including CD4, viral load, and ART duration. Similar results were found for the other CAD indices of CAD (CAC: aOR=1.72; 95% CI: 1.42–2.08; p < 0.001; VP: aOR=1.34; 95% CI: 1.10–1.63; p=0.003; Leaman score >5: aOR 2.0; 95% CI: 1.56–2.57; p < 0.001). PCAT density was increased among REPRIEVE participants compared to FHS controls (-87.9±10.2 vs. -90.8±9.1 HU; p < 0.01) and remained significant in least squares random effects modeling (p = 0.01).

Conclusion: Among PWH in REPRIEVE, a large primary CVD prevention cohort, increased PCAT density is independently associated with prevalence and severity of CAD. Moreover, PCAT density was higher among PWH in REPRIEVE than non-HIV controls. These data provide novel evidence linking increased pericoronary inflammation to CAD in PWH. Understanding the mechanisms and longer-term consequences of increased pericoronary inflammation may help guide preventive strategies for improved cardiovascular health in PWH.

Pericoronary Adipose Tissue Density in REPRIEVE

PERICORONARY ADIPOSE TISSUE DENSITY AND SUBCLINICAL CORONARY ARTERY DISEASE IN HIV

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REPRIEVE Study Team

Background: Skeletal muscle quality and mass are critically important for maintaining physical function during advancing age. We leveraged baseline data from REPRIEVE to evaluate whether paraspinal muscle density (MD) and muscle area (MA) are associated with cardiac or physical function outcomes in people with HIV (PWH).

Methods: REPRIEVE is a double-blind randomized trial evaluating the effect of pitavastatin for primary prevention of coronary artery disease (CAD) in PWH. This cross-sectional analysis focuses on participants who underwent coronary CT at baseline. Lower thoracic paraspinal MD (Hounsfield units, HU) and MA (cm²) were assessed on non-contrast CT images. MA was divided by height to allow examination of associations across various body sizes (MA/HT). Physical function was standardized by maximum performance. Associations between muscle measures (risk factors) and baseline coronary CT and physical function measures (outcomes) were evaluated using log-binomial regression and linear regression models adjusted for age, sex, and body mass index (physical function only).

Results: Of 805 PWH, 708 had paraspinal measurements. Median age was 51 (IQR: 43–55) years; 17% were natal female, 53% White, 36% Black, and 25% Hispanic. The median MD was 41 (31, 49) HU in males and 30 (16, 39) HU in females, and MA/HT 13.2 (9.4, 16.3) cm²/m in males and 9.7 (7.5, 13.2) cm²/m in females. In adjusted analyses, higher MD (less fat) was associated with lower prevalence of coronary artery plaque, lower prevalence of coronary artery calcium score >0, and lower prevalence of high plaque burden (Leaman score >5; p = 0.06), while MA/HT was not associated with cardiac measures (Figure panel a). Among the 139 with baseline physical function measures, greater MA/HT was associated with better performance on a short physical performance battery and grip strength, while no associations between MD and physical function measures were apparent (Figure panel b).

Conclusion: Higher MD (less fat) was associated with lower prevalence of CAD, while higher MA was associated with better physical performance in PWH. Whether changes in muscle fat or area (especially if associated with pitavastatin) are associated with changes in CAD or improved physical performance will be evaluated as part of the REPRIEVE longitudinal analyses.
Methods: We obtained coronary arteries with varying stages of atheroma from deceased PWH (n=13) and matched HIV-negative donors (n=15). We measured virus copies within coronary arteries using droplet digital polymerase chain reaction (ddPCR) for HIV, cytomegalovirus (CMV), and human endogenous retrovirus (HERV-K). We performed an unbiased analysis with a spatial whole transcriptomic approach (n=7 per group) and defined specific immune markers by targeted spatial transcriptomics, proteomics, and immunohistochemistry. Comparisons of immunohistochemistry results used Mann-Whitney U tests, and comparisons of multiple regions used linear models for microarray data (LIMMA) with Benjamin Hochberg correction.

Results: Among coronary arteries matched by plaque type (pathological intimal thickening, early and late atheroma), plaque area, and stenosis (Figure 1A-B), we detected viral RNA sequences for HIV in 77% (10/13) of coronary arteries from PWH, while CMV was detected in 46% (6/13) of PWH and 53% (8/15) of controls. Coronary arteries from all donors had detectable HERV-K. The RNA transcriptomes differed by HIV status with enrichment of genes in several inflammatory pathways including the interferon signaling and nonsense-mediated decay pathway in PWH (Figure 1C). Partial Spearman rank correlation adjusted for plaque type in the PWH showed a significant relationship between HIV copies with STING, CSF3, and VCAM1 protein expression.

Conclusion: The presence of HIV within the coronary arteries alters the immune environment with stimulation of several pathways such as interferon-gamma and the non-sense mediated decay pathway. Notably, the presence of other chronic viruses such as CMV or HERV-K does not appear to differ by HIV status. HIV may be an important accelerator of atherosclerosis and increased CVD risk in PWH. Further studies are underway to establish whether intact virus, viral sequences or modified host DNA stimulate inflammation within coronary plaques.

651 HIGH PLASMA APOE IN PEOPLE WITH HIV COMPARED WITH UNINFECTED CONTROLS
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Background: High levels of apolipoprotein E (apoE) in plasma are associated with increased risk of cardiovascular disease and all-cause mortality in the general population. APOE gene polymorphisms accounts for 15–25% of variability in plasma apoE, but plasma apoE still vary between individuals with the same APOE genotype. Our aims were to assess if HIV is an independent risk factor for high plasma apoE and to determine HIV related risk factors for high plasma apoE.

Methods: We included 661 people with HIV (PWH) frequency matched 1:1 on age and sex with uninfected controls from the general population. Only people of Danish ancestry were included to minimize differences in APOE genotypes. Both PWH and controls underwent physical examination, filled out extensive questionnaires and had blood samples drawn according to the same study protocol.

Results: Mean age was 52 years and 11% were female in both groups. Median plasma apoE was 49.0 mg/L in PWH and 43.3 mg/L in controls. High apoE was defined as above 66.2 mg/L.

In adjusted linear models PWH had 6.6 mg/L higher plasma apoE compared with controls (95%CI: 5.1 to 8.1 mg/L, p < 0.001). PWH also had higher odds of high plasma apoE with adjusted odds ratio (OR) of 2.55 [1.63, 3.99], p < 0.001. In age-adjusted analyses stratified by sex the estimated ORs for each sex were similar (OR 2.03 [1.35, 3.04], p < 0.001 for males and OR 2.02 [0.69, 5.89], p=0.20 for females).

In PWH, previous AIDS defining disease was associated with 4.50 mg/L higher plasma apoE [1.52, 7.48 mg/L, p=0.003 in sex- and age-adjusted analysis. Previous AIDS was also associated with higher odds of high apoE (OR 1.78 [1.03, 3.06], p=0.04) in unadjusted analysis, but not in adjusted models. CD4 nadir, detectable viral load, and exposure to old generation anti-retroviral therapy was not associated with plasma apoE levels.

Conclusion: PWH had higher concentrations of plasma apoE compared with uninfected controls even after adjusting for plasma lipids. Previous AIDS defining disease was associated with higher plasma apoE. Further studies are needed to elucidate the clinical impact of high plasma apoE in PWH.

652 PERFORMANCE OF LDL CHOLESTEROL POLYGENIC RISK SCORE IN INDIVIDUALS WITH HIV INFECTION
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Background: Coronary artery disease (CAD) is a leading cause of death among the 37 million people with HIV (PWH) globally. Available cardiovascular disease (CVD) risk predictors underperform in this population. While alleles that influence LDL-C concentration summed as a polygenic risk score (PRS) predict LDL-C and CAD in the general population, their influence among PWH is not well understood.

Methods: A new PRS for LDL-C was constructed using PRS-CS, incorporating the weighted effects of over 1 million single nucleotide polymorphisms associated with LDL-C from over 1.9 million individuals without HIV from the Global Lipids Genetics Consortium, European Network for Genetic and Genomic Epidemiology, Biobank Japan, Genes and Health Study, and Million Veterans Program. Scores were trained in the UK Biobank then calculated using TOPMed-imputed genotypes from a global cohort of ART-treated PWH with low-to-moderate CVD risk enrolled in Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE). Genotyping was performed on Illumina Infinium HTS. Linear and logistic regressions were performed to predict baseline lipid profiles in 4177 genotyped participants and markers of subclinical CAD including presence of plaque, calcification, or stenosis in a subset of 639 participants who received coronary computed tomography angiography (CT sub-study).

Results: Among a cohort of 4177 REPRIEVE participants (mean [SD] age 49.8 [6.3] years, 36.1% female; 56.5% African, 12.6% East Asian, 21.2% European, 9.6% South Asian ancestries), LDL-C PRS was correlated with fasting LDL-C (r = 0.33, p < 1E-97; Figure 1A), total cholesterol (r = 0.29, p < 1E-72), and oxidized LDL-C (r = 0.15, p < 1E-3). The mean [SD] LDL-C was 107.2 [30.7] mg/dL. Each standard deviation of the LDL-C PRS was associated with a 10.2 (95%CI 9.3 – 11.1, p < 0.001) mg/dL increase in LDL-C. The difference in LDL-C between the top and bottom one percentiles of the LDL-C PRS in REPRIEVE was 56.2 mg/dL (Figure 1B).

Similar association results were identified in subgroups stratified by...
self-reported ancestry and sex. Among 639 participants in the CT sub-study, we did not detect an association between LDL-C PRS and markers of subclinical CAD.

**Conclusion:** Among ART-treated PWH in REPRIEVE, a newly developed PRS for LDL-C was associated with levels of LDL-C, total cholesterol, and oxidized LDL-C, but not with subclinical CAD. Future analyses within REPRIEVE will assess whether LDL-C PRS predicts major adverse cardiovascular events in this at-risk population.

Figure 1

**653** **CHOLESTEROL EFFLUX IS REDUCED IN ART TREATED PWH BUT ASSOCIATED WITH SUBCLINICAL CAD**

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**Background:** Impaired cholesterol efflux (MCE) from monocytes may lead to formation of lipid-laden ‘foam cells’ which are implicated in atherosclerotic coronary artery disease (CAD). Monocyte cholesterol efflux (MCE) may be impaired in people with HIV (PWH) with evidence of persistent abnormalities after antiretroviral therapy (ART) initiation, however, it is unclear whether this is associated with subclinical CAD.

**Methods:** We examined MCE ex vivo in monocytes isolated from PWH, virally suppressed on ART and propensity score (incorporating traditional CAD risk factors) matched uninfected controls. We measured total MCE as well as ABCA1-dependent MCE by exposing isolated monocytes, pre-incubated with LDL cholesterol, to Apolipoprotein A1 at 6 and 24 hours incubation, with MCE calculated as the ratio of measured intracellular and extracellular cholesterol at each time-point. Associations with MCE and coronary plaque phenotypes, as measured by coronary computer tomographic angiography (CCTA), were assessed using logistic regression. Data are median unless stated otherwise.

**Results:** Ninety five participants [age 50 (46, 56) years, 72% male, 76% Caucasian, 47 (49.5%) PWH] were included. Risk factors for CAD were evenly distributed across groups with the exception of HDL cholesterol concentration [PWH: 1.3 (1.0, 1.3); controls:1.4 (1.1, 1.7) mmol/l, p = 0.02], and statin use [PWH: 24 (51.1%), controls:6 (12.5%), p = 0.001]. LDL-C was significantly less ABCA1-dependent MCE at 6 (T6a) hours [PWH 0.75 (0.6,1.0); controls 1.04 (0.7 ,1.33), p =0.01], which did not persist at 24 hours [PWH 1.23 (0.8, 1.6), controls: 1.39 (1.0, 1.8), P =0.24]. We did not observe differences in other MCE measures.

**Conclusion:** PWH on long term ART had reduced ABCA1 dependent MCE compared to CAD risk matched controls. That MCE (T6a) was associated with non-calcified plaque gives further insights into the role of Monocyte/macrophages in CAD pathogenesis and warrants further investigation into potential mechanisms underlying the increased risk of CAD in PWH.

**654** **SMOKING CESSATION AND SHORT-TERM VASCULAR IMPROVEMENT IN A COHORT OF PEOPLE WITH HIV**

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1Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil, 2Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 3Institute of Clinical Pharmacology, Rio de Janeiro, Brazil

**Background:** Smoking is highly prevalent in people living with HIV/AIDS (PLHA) producing detrimental effects in different organs and leading to illness. There is limited evidence about pharmacological interventions for treating nicotine dependence in PHLA. We examined if Nicotine replacement therapy (NRT) is an option for smoking cessation and ameliorates vascular health in this specific population.

**Methods:** From December 2019 to April 2022, we prospectively enrolled PHLA who were actively smoking in our center. The primary outcome of interest was to assess the effect of NRT plus counseling on smoking cessation and endothelial function measured by brachial artery flow-mediated dilatation (FMD). Statistical analysis evaluated the change in %FMD (Δ%FMD = %FMD at week 12 - %FMD at baseline) to test the hypothesis that Δ%FMD would improve among participants who quit smoking compared to those who relapse. To confirm the results, we have run multiple linear regression to account for classical cardiovascular (CV) confounders. Results are presented in medians (interquartile ranges) and percentages.

**Results:** We included 117 participants with median age of 45.5 years (IQR= 36.4-54.8), 29 (25.4%) had hypertension, 10 (8.8%) had diabetes and 33 (28.4%) had dyslipidemia, almost half were smoking 20+ cigarettes/day (41.4%). Individuals were living with HIV for a median of 10.9 years (5.6-17.6) and were on antiretroviral therapy for 8.6 years (3.7-13.6) with median Nadir of CD4 of 307 (153–490.5). Baseline of median brachial artery diameter was 3.6 mm (IQR= 3.2-4.1). Unadjusted analysis showed that years of smoking, younger age and white race were associated with poor %FMD (75th per centile). After 12 weeks 44.4% participants quit smoking. Comparison of Δ%FMD change from baseline to week 12 showed that among participants adherent to therapy, there has been an increase in Δ%FMD when compared to those who relapsed (1.17% [0.29-2.98] vs -0.19% [-1.9-0.91], p < 0.001). After adjustment for CV factors, multiple linear regression showed that participants who quit smoking present a mean of 2.54 (p = 0.001) points increase in Δ%FMD in comparison to those who continued to smoke.

**Conclusion:** This study provides evidence that a strategy of NRT and counseling is effective for smoking cessation in PHLA leading to an improved vascular health in a short period of time. This reinforces the importance of the widespread anti-tobacco programs in HIV clinics and the expected impact lowering the incidence of future cardiovascular events.

![Figure 1](image1.png)

**Figure 1:** (a) Monocyte Cholesterol Efflux at 6 hours incubation with and without ApoAI (b) associations with subclinical CAD.

![Figure 2](image2.png)

**Figure 2:** Plot of individuals %FMD in Week 1 and Week 12 according to NRT.
655 AORTIC ANEURYSMS AND MARKERS OF ENDOTHELIAL AND PLATELET ACTIVATION IN PLWH
Sylvester Gronbaek1, Julie Hogh1, Michael Pham1, Andreas D. Knudsen1, Andreasuchs1, Sisse Ostrowski2, Thomas Lars Benfield3, Klaus Kofoed4, Susanne Dam Poulsen1
COCOMO study
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Background: People living with HIV (PLWH) have more than four times higher odds of aortic aneurysms (AA) compared with the uninfected population. There are currently no studies of biomarkers of AA in PLWH. We aimed to investigate if sCD40L, D-dimer, syndecan-1, and thrombomodulin were associated with AA in PLWH.

Methods: From the Copenhagen Comorbidity in HIV Infection (COCOMO) study, PLWH ≥40 years of age with an available contrast-enhanced CT scan and available biomarker analyses were included. AA was defined according to guidelines. The biomarkers were analysed on thawed plasma using Luminex®, a multiplex custom-designed assay. For each biomarker, levels in the upper quartile were defined as “high concentration”. For D-dimer, the cut-off was defined as the lower limit of detection. Using adjusted logistic and linear regression models, we analysed associations between AA and sCD40L, D-dimer, syndecan-1, and thrombomodulin, respectively, in PLWH.

Results: We included 571 PLWH in whom 43 AA were found in 39 (6.8%) individuals. The median (IQR) age was 52 years (47-60), 88% were male, median (IQR) time since HIV was diagnosed was 15 years (8-23), and 565 (99%) were currently on antiretroviral treatment. High concentration of sCD40L was associated with lower odds of AA (adjusted odds ratio, aOR: 0.23 (95% CI 0.07-0.78; P = 0.019)), and high concentration of D-dimer was associated with higher odds of AA (aOR: 2.22 (95% CI 1.02-4.85; P = 0.045)). Syndecan-1 and thrombomodulin were not associated with AA (P = 0.52 and P = 0.47, respectively). In linear regression, high concentration of sCD40L was associated with 0.46 mm (95% CI 0.14 to 0.78 mm) smaller suprarenal aorta; high D-dimer was associated with 0.97 mm (0.36 to 1.54) larger infrarenal aorta; and high concentration of thrombomodulin was associated with 1.11 mm (0.30 to 1.92) larger ascending aorta, 0.68 mm (0.14 to 1.21) larger suprarenal aorta and with 0.89 mm (0.62 to 2.00) larger infrarenal aorta.

Conclusion: Prevalence of AA is high in PLWH. sCD40L was associated with lower odds of AA and D-dimer was associated with higher odds of AA in PLWH calling for further investigations into specific biomarkers to aid early diagnosis of AA in PLWH.

Aortic diameters and markers of endothelial and platelet activation, and haemostasis in PLWH

656 BMI MEDIATES THE LINK BETWEEN HIV-RELATED FACTORS AND HYPERTENSION BUT NOT DIABETES
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Background: HIV infection has been associated with an increased cardiometabolic risk profile, due to the HIV virus and the effects of ART. However, the nature of the causal pathway is unclear. This study aims to assess the mediating effect of body mass index on the association between HIV-related factors (ART exposure, CD4 count and viral load) and hypertension and diabetes mellitus (hereinafter referred to as diabetes) among people living with HIV/AIDS in a Sub-Saharan Africa setting.

Methods: This cross-sectional study included 14,119 consenting adults enrolled in the International Epidemiology Databases to Evaluate AIDS (IeDEA) in Cameroon, between 2016 and 2021. Hypertension was defined as Systolic BP (SBP) ≥140/90 mmHg and/or current use of antihypertensive medication while diabetes was defined as an elevated Fasting Blood Sugar (FBS) ≥126 mg/dL or use of antidiabetic medications. We conducted a causal mediation analysis through a nonparametric bootstrapped method using the “mediation” package for causal mediation analysis in R. The exposures were ART exposure (binary), viral load (continuous) and CD4 count (continuous), assessed individually. The mediator was BMI (continuous) and the outcomes were either diabetes (binary) or hypertension (binary). We adjusted for the confounders, age, sex, and smoking (Figure 1).

Results: The study population was made up of 9,177 (65%) women, 1,694 (12%) were obese (BMI ≥ 30 kg/m²) and 8,869 (63%) had been exposed to ART. The median(IQR) CD4 count was 373.0 (199.2, 565.0) cells/mm³ and the median (IQR) viral load was 1.6 (0.0, 1.9) log₁₀ copies/mL. BMI significantly mediated the total effects of ART exposure (estimate: 0.02, 95% CI: 0.015 to 0.025, 43.9% mediated), viral load (estimate: -0.003, 95% CI: -0.003 to 0.00, 26.9% mediated) and CD4 count (estimate: 5.23 x 10⁻¹⁵, 95% CI: 4.16 x 10⁻¹⁰ to 0.00, 68.7% mediated) on hypertension. However, the mediating effect of BMI on the relationship between ART use, viral load and CD4 count, and diabetes was not observed.

Conclusion: These findings suggest that BMI partially mediates the association between ART use, CD4 count and viral load and hypertension but not diabetes, among people living with HIV when controlled for traditional cardiovascular risk factors. This study underscores the importance of BMI as a useful measure for assessing hypertension and diabetes among people living with HIV. Mediation model for the association between HIV related factor (ART exposure, CD4 count or viral load) and hypertension or diabetes; with body mass index (BMI) as the mediator and age, sex and smoking as confounders.

657 RACIAL AND HIV DISPARITIES IN HYPERTENSION TREATMENT CASCADE AMONG WOMEN
Jessica Blair1, Mirjam Colette-Kempf1, Jodie Dionne-Odom, Zoeania Causey-Prutt1, Jenni Wise, Elizabeth Jackson, Paul Muntner1, Jorge R. Kizer2, Margaret Fischl1, Igbo Ofotokun1, Adaora Adimora1, Stephen Gange1, Emily B. Levitan1
1University of Alabama at Birmingham, Birmingham, AL, USA, 2Albert Einstein College of Medicine, Bronx, NY, USA, 3University of California San Francisco, San Francisco, CA, USA, 4University of Miami, Miami, FL, USA, 5Emory University, Atlanta, GA, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 7The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Management of hypertension has emerged as a priority among women living with HIV (W-LWH). Our goal was to evaluate cross-sectional associations of race/ethnicity and HIV status with prevalence, awareness, treatment, and control of hypertension among women participating in the Women’s Interagency HIV Study (WIHS).

Methods: This study included women who were recruited into WIHS Southern sites between 2013 and 2015. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, self-report, or use of anti-hypertensive medication. Awareness was self-reported as ever having hypertension and treatment was reported use of anti-hypertensive medication for hypertension. Blood pressure control was defined as < 140/90
mmHg as aligned with clinical guidelines when baseline data were collected. Prevalence ratios (PRs) for each hypertension outcome were estimated using Poisson regression models with robust variance estimates adjusted for sociodemographic, behavioral, and clinical risk factors.

**Results:** Among 712 women participating in WHS Southern sites, 602 (84%) were non-Hispanic (NH) Black, 70 (10%) were NH White, and 40 (6%) were Hispanic, including 493 (69%) WLHIV. The average age was 43 years and 401 (56%) had hypertension. Eighty-three percent of women with hypertension were aware of their diagnosis. Of those aware, 83% were currently taking anti-hypertension medication and 63% of women who were treated for hypertension had documentation of controlled hypertension. We found that NH White and Hispanic women had lower prevalence of hypertension compared to NH Black women [PR 0.70 (95% CI 0.54–0.90) and PR 0.52 (95% CI 0.32–0.85), respectively, p < .0001]. Additionally, Hispanic women had better controlled hypertension compared to NH Black women [PR 2.16 (95% CI 1.54–3.01), p < .0001]. WLHIV who had hypertension were more likely to be on anti-hypertension medication compared to women without HIV [PR 1.19 (95% CI 1.01–1.40), p = .0345] (Figure 1).

**Conclusion:** In this study population of women living with and without HIV in the U.S. South, prevalence of hypertension was lowest among Hispanic women and highest among NH Black women. Control of hypertension was lowest among NH Black women. Awareness and treatment were similar among race/ethnicity. Women without HIV were less likely to be on an anti-hypertension medication compared to WLHIV although prevalence of hypertension was similar between both groups.

Proportion of hypertension outcomes by race/ethnicity and HIV status

**Figure 1.** Proportions of hypertension outcomes by race/ethnicity and HIV status

### 659 MARIJUANA USE AND THE RISK OF INCIDENT VENOUS THROMBOEMBOLISM IN PEOPLE WITH HIV

**Lara Haidar**1, Robin Nance2, Stephanie Ruderman2, Alekhyia Lavu, Laila Aboulatta,1 Payam Peymani3, Andrew Hahn2, Amanda Willig4, Katerina Christopoulos5, Jeannie Keruly6, Kristina Crothers7, Joseph Delaney7, Heidi Crane1, Sherif Eltonisy2

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**Background:** People with HIV (PWHL) are at an increased risk of venous thromboembolism (VTE). Among PWHL, marijuana use is common. Marijuana has been shown to display both procoagulant and anticoagulant effects on the blood, however its effect on VTE in PWHL has not been evaluated. We aimed to assess whether there is an association between marijuana use and incident VTE among PWHL.

**Methods:** We conducted a cohort study using data from the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS), a US-based, multisite cohort of PWHL. Marijuana use was obtained from a clinical assessment of patient reported outcomes on substance use collected as part of care using the modified ASSIST instrument. VTEs were assessed using multiple ascertainment criteria and then centrally adjudicated by at least 2 expert physician reviewers. Only first VTEs were included among PWHL who have had more than one. Cox models were used to determine the association of incident VTE with marijuana use. Models were adjusted for age, sex, other substance use, CD4 cell count, HIV viral load, diabetes, hypertension, dyslipidemia, chronic kidney disease (CKD [eGFR< 30]), Hepatitis C (HCV), and Hepatitis B (HBV) co-infection.

**Results:** Among 12,515 PWHL in care between 2009 and 2019 at 6 CNICS sites across the US, 213 (1.7%) experienced a VTE. Mean follow up was 4.5 years, mean age was 44 years, 17% were female, 45% were white, and 32% reported current marijuana use (defined as any use in the past 3 months). Around 18% had dyslipidemia, 16% had HCV co-infection, 9% had diabetes, and 1% had CKD. The mean CD4 count was 532 cells/mm$^3$ and 19% had a viral load >400 copies/mL. In adjusted models, former (adjusted hazard ratio [aHR] 0.83, 95% CI 0.57-1.20) and current (aHR 0.78, 95% CI 0.51-1.33) marijuana use were not associated with a significant increase in VTE incidence compared to never users. Furthermore, no association was observed between frequency of marijuana use and risk of incident VTE, suggesting there is no dose-dependent increase in VTE risk.

**Conclusion:** Among PWHL there seems to be no evidence of increased VTE risk with the use of marijuana, and so VTE risk mitigation does not need to specifically consider marijuana use.

Association between marijuana use and VTE in adjusted Cox models (n=12,515)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adjusted HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marijuana use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>0.83 (0.57-1.20)</td>
<td>0.324</td>
</tr>
<tr>
<td>Current</td>
<td>0.76 (0.51-1.13)</td>
<td>0.178</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of marijuana use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Infrequent user (no use)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>1.35 (0.84-2.16)</td>
<td>0.215</td>
</tr>
<tr>
<td>Current</td>
<td>1.62 (1.17-2.24)</td>
<td>0.020</td>
</tr>
<tr>
<td>Weekly</td>
<td>1.17 (0.81-1.72)</td>
<td>0.630</td>
</tr>
<tr>
<td>Current Daily</td>
<td>0.81 (0.55-1.17)</td>
<td>0.592</td>
</tr>
</tbody>
</table>

Note: Data are adjusted for age, sex, CD4 cell count, HIV viral load, diabetes, hypertension, dyslipidemia, chronic kidney disease (CKD [eGFR< 30]), Hepatitis C (HCV), Hepatitis B (HBV) co-infection, marijuana use, and VTE venous thromboembolism.
**650**

LOW CD4 NADIR AT HIV DIAGNOSIS ASSOCIATES WITH INCREASED RISK OF CLONAL HEMATOPOIESIS

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**Background:** People with HIV (PWH) have excess risk of cardiovascular disease (CVD), which is thought to be related to dysregulated inflammation. Analogously, clonal hematopoiesis of indeterminate potential (CHIP) activates an inflammatory cascade in infiltrating monocytes to promote atherosclerosis. CHIP prevalence is known to be increased in PWH, but whether HIV-specific risk factors are associated with CHIP prevalence or CHIP gene distribution is not known.

**Methods:** Participants were from a global cohort of ART-treated PWH with low-to-moderate CVD risk enrolled in Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) with whole exome sequencing (WES) data available. CHIP was defined by the presence of pathogenic somatic variants in genes previously implicated in hematologic cancers with a variant allele fraction (VAF) ≥2% but without hematologic cancer or other non-neoplastic clonal disease. Multiple logistic regression models adjusting for age, sex and race were used to associate CHIP with HIV-specific risk factors.

**Results:** Among 4486 REPRIEVE participants (mean [SD] age 49.9 [6.4] years, 36.8% female; 46.6% Black, 23.4% Asian, 23.8% White self-reported race), CHIP prevalence was 3.7% among PWH. The most common driver variant was TP53 (53.3%), TET2 (15.6%), DNMT3A (9.0%), ASXL1 (4.0%), and TSPAN3 (4.0%). After adjustment for age, sex and race there were no associations between CHIP and enrolment CD4+ cell count, ART regimen, or specific ART exposures (abacavir, protease inhibitors, thymidine, or TDF (tenofovir disoproxil fumarate)). The odds of CHIP were higher with longer total ART duration, but not significantly associated with each outcome, while NEFA, hsCRP and sST2 levels were significantly associated with each outcome.

**Conclusion:** We identified previously unreported associations of several key biomarkers with cardiac dysfunction, including more pronounced associations of troponin I among PWH. Associations of circulating biomarkers with cardiac dysfunction, per Z-score unit.

**661**

CIRCULATING BIOMARKERS AND CARDIAC DYSFUNCTION IN WOMEN WITH OR AT RISK FOR HIV

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**Background:** People with HIV are at greater risk for cardiovascular disease compared with those without HIV, and this risk may be further elevated in women with HIV (WWH). We examined 8 circulating cardiac, metabolic or inflammatory biomarkers in women with or at risk for HIV with echocardiographic data, and assessed whether these markers are associated with cardiac dysfunction.

**Methods:** Eligible participants from 3 Women's Intergency HIV Study (WWHS) sites underwent transthoracic echocardiography between 2014-2018 and had the following serum biomarkers measured: NT-proBNP, high-sensitivity troponin I (hsTnI), soluble ST2 (sST2), non-esterified fatty acids (NEFA), C-reactive protein (hsCRP), interleukin-6 (IL-6), soluble CD14 (sCD14) and growth-differentiation factor-15 (GDF-15). Outcomes included left ventricular (LV) systolic dysfunction (LV ejection fraction < 54%), isolated LV diastolic dysfunction (AASE 2016 definition) and pulmonary hypertension (peak tricuspid regurgitation velocity > 2.8 m/s). Biomarkers were log-transformed and Z-score standardized, and modified Poisson regression models produced prevalence ratios (PRs) for associations with each outcome, adjusted for demographic, behavioral and cardiometabolic factors.

**Results:** Data were available for 709 women (68% WWH, median age 52 years, 65% black, 22% Hispanic, 10% smokers). Among WWH, median CD4+ cell count was 649 cells/µL (IQR 442-689), 93% reported ART use and 69% had HIV viremia < 20 cp/mL. Prevalence was 6% in WWH and 4% without HIV for LV systolic dysfunction, 5% and 8% for isolated LV diastolic dysfunction, and 12% and 9% for pulmonary hypertension. Compared with women without HIV, WWH had higher levels of sCD14 (median 1.792 vs 1.539 ng/mL) and GDF-15 (median 1.069 vs 0.679 pg/mL, both p < 0.001), with a stepwise gradient by CD4+ cell category. In adjusted analyses (Table), higher NT-proBNP levels were significantly associated with each outcome, while NEFA, hsCRP and sST2 levels were associated with LV systolic dysfunction only, and GDF-15 with pulmonary hypertension only. hsTnI was associated with both isolated LV diastolic dysfunction and pulmonary hypertension, and these associations were more pronounced among WWH than women without HIV (e.g., pulmonary hypertension: WWH PR 1.88, 95% CI 1.35-2.62; without HIV PR 1.46, 95% CI 0.95-2.24).

**Conclusion:** We identified previously unreported associations of several key cardiac biomarkers with cardiac dysfunction, including more pronounced associations of troponin I among WWH. Associations of circulating biomarkers with cardiac dysfunction, per Z-score unit.

**662**

CD2/LFA-3 AXIS COSTIMULATION OF CD57+ CD28- CD8 T CELLS IN HIV AND ATHEROSCLEROSIS

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**Background:** Immune activation plays a critical role in people with HIV (PWH) who have an increased risk of cardiovascular disease (CVD), even after controlling for known CVD risk factors. Latent cytomegalovirus (CMV) infection is associated with increased CVD risk for people with and without HIV (PW0H). T cells from CMV+ individuals are enriched with an inflammasome CCR5+CD57+CD28- phenotype, suggesting they may require a pathway separate from the canonical CD28 pathway for optimal costimulation. We have shown that CMV coinfection in PWH promotes vascular homing and activation of inflammatory CD4 T cells through the CD2/LFA-3 axis, but a role for CD2/LFA3 costimulation of CD57+CD28-CD8 T cells in CMV+ PW0H has not yet been described.

**Methods:** Cells from PW0H (n=46, 17% female), PW0H (n=15, 67% female), and from atherosclerotic plaques of PW0H (n=8, 25% female) were analyzed for expression of CD2, CD28, CD57, CCR5 and other markers by flow cytometry and compared by CMV status. Cells were stimulated in vitro with anti-CD3 plus either anti-CD28 or plate-bound LFA-3 for up to 7 days and measured for viability, cell cycling/proliferation, cytokine and lytic granule expression, glucose uptake, mitochondrial biogenesis, and Bcl-2 expression. Immunofluorescence imaging for LFA-3 was performed on aortic tissues from SIV/SIVH-infected rhesus monkeys.
macaques and uninfected animals, and from PWoH ± atherosclerosis. LFA3 gene expression was assessed by real-time RT-PCR in human aortic endothelial cells stimulated with TNF in vitro.

**Results:** CD2 expression on vascular-homing CXC3CR1+CD57+CD28- CD8 T cells was increased on cells from CMV+ PWH. In vitro, costimulation with LFA-3 potently enhanced TCR-mediated cytokine (IFNγ, TNF, IL-2, MIP-1β) and lytic granule production by these inflammatory CD8 memory T cells compared to CD28 costimulation. Proliferation, glucose uptake, mitochondrial biogenesis, and Bcl-2 expression were similarly upregulated. Finally, LFA-3 protein was highly expressed in aortas of rhesus macaque HIV model and in atherosclerotic plaques of PWH. Additionally, human aortic endothelial cells increased LFA3 gene expression upon TNF exposure.

**Conclusion:** Our data support a model in which CMV infection enhances CD2 expression on highly proinflammatory inflammatory CD8 T cells, which can then be stimulated by LFA-3 expressed in the inflamed vasculature, even in the absence of CD28 costimulation, highlighting a potential therapeutic target in atherosclerosis development and progression, especially for PWH.

### 663 ANTIRETROVIRAL THERAPY AMELIORATES SIV-ASSOCIATED MYOCARDITIS IN THE HEART

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**Background:** With introduction of antiretroviral therapy (ART), human immunodeficiency virus (HIV) progressed to a chronic inflammatory disease with accelerated, subclinical end-organ damage, specifically cardiovascular disease (CVD). People with HIV (PWH) have higher risk and mortality from CVD, such as atherosclerosis, diastolic dysfunction, and heart failure. Several recent clinical reports show maladaptive concentric hypertrophy, increased fibrosis, and left ventricle dysfunction in PWH, and as such, investigation into molecular mechanisms promoting pathological changes in the heart is necessary.

**Methods:** We utilize the highly translatable simian immunodeficiency virus (SIV)-infected rhesus macaque model to identify changes in the myocardium with and without ART. We performed total RNA Seq on left ventricle tissue from uninfected animals (n=3), SIV-infected animals (n=4), and SIV-infected animals receiving a clinically relevant ART regimen (n=4).

**Results:** SIV infection led to high plasma viral load, but little to no SIV RNA was detectable in the left ventricle, shown by minimal of SIV RNA+ cells in the heart and no SIV sequences identified from RNA-Seq. SIV infection produced a highly inflammatory reaction in the heart, predominated by interferon (IFNβ, IFNγ, IL-12, IL-15, IL-28A, IL-4, IL-6, IL-15, IL-28B, TNFα, IL-27), and interferon response in the heart; however, SIV-infected animals receiving ART exhibited decreased expression of integral genes directly involved in fatty acid (FA) metabolism, carnitine shuttling, and beta oxidation (C3, D3βH, DBI, SC52A0, CPT1A, CPT1B, CPT2, UC2, ACAC1, ASC1L1, ACD, DHAD1, 2).

**Conclusion:** These data provide an important addition to the understanding of inflammatory and metabolic changes in the heart during SIV infection and treatment with ART. Additionally, deficits in FA metabolism and beta oxidation in ART animals could stem from either prolonged low-grade infection, off-target effects of ART, or synergistic effects of infection with ART. Future studies should address the long-term effects of HIV infection with ART on the heart for a clinical understanding of the predisposition of PWH to developing early CVD.

### 664 EFFECT OF NICOTINE RECEPTOR AGONISTS ON INFLAMMATION AND RISK OF CHD AND DEATH IN HIV

Hilary A. Tindle, Debbie M. Cheng1, Natalia Gnatienko1, Elena Blokhina1, Tatiana Yaroslavtseva1, Sally Bendick1, Leah Forman1, Judith A. Halin1, Kuki So-Armah1, Michael D. Stein1, Kendall Bryant1, Dmitriy Liszowskij, Evgeny Krupitsky1, Jeffrey H Samet1, Matthew Freiberg2

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**Background:** People with HIV (PWH) have elevated systemic inflammation, even after HIV viral suppression, which is exacerbated by risky drinking and smoking. Nicotine receptor agonists promote smoking cessation, may reduce alcohol consumption, and may directly reduce inflammation via cholinergic stimulation. If varenicline, cytisine, or nicotine replacement therapy (NRT) differentially reduced biomarkers of inflammation and risk for coronary heart disease (CHD) and mortality independent of abstinence from smoking or alcohol this could alter treatment guidelines.

**Methods:** We conducted a four-arm randomized, double-blinded, placebo-controlled trial among 400 heavy drinking (> 5 heavy-drinking days in the past month) and daily smoking PWH in St. Petersburg, Russia from 2017-2020. All participants received one active medication and one placebo: Arm 1: varenicline + placebo NRT; Arm 2: placebo varenicline + NRT; Arm 3: cytisine + placebo NRT; and Arm 4: placebo-cytisine + NRT. Treatment regimens ranged from 25 days (cytisine) to 12 weeks (varenicline). Outcomes, assessed at 3 months, were: high sensitivity c-reactive protein (hsCRP; µg/ml), IL-6 (pg/ml), Reynolds Risk Score (10-year % risk CHD), and VACS Index (5-year all-cause mortality risk). We performed linear regression analyses controlling for randomization stratification factors (alcohol consumption, average daily cigarettes, current antiretroviral therapy).

**Results:** Randomized groups were similar on baseline characteristics: 66% male, mean age 39 years, mean CD4 count 391 cells/mm3, and 57% undetectable HIV viral load. There were no significant differences in the three main comparisons for hsCRP at 3 months: arm 1 vs. 2 (adjusted ratio of means [AROM] 1.25; 95%CI 0.61, 2.54, p=0.73), arm 3 vs. 4 (AROM 1.13; 95%CI 0.55, 2.35, p=0.73), or arm 1 vs. 3 (AROM 1.21; 95%CI 0.58, 2.49, p=0.73) (Table). Similarly, 3-month IL-6, Reynolds Risk Score, and VACS Index did not differ by group (Table). In exploratory analyses, participants who quit drinking and smoking appeared to have lower 3-month hsCRP, IL-6, and VACS scores compared to those who continued both behaviors (Table).

**Conclusion:** Among PWH with heavy drinking and smoking, biomarkers of inflammation and risk of CHD and mortality did not differ by treatment group. These results do not support the hypothesis that nicotine receptor partial and full agonists lower levels of inflammation independent of smoking or alcohol cessation.

**Table 1. Effects on Inflammatory Biomarkers, Cardiovascular Risk and Mortality Risk Among PWH with HIV (PWH) with Risky Drinking and Daily Smoking in Russia**

<table>
<thead>
<tr>
<th>Main Outcome</th>
<th>Arm 1 vs. 2</th>
<th>Arm 3 vs. 4</th>
<th>Arm 1 vs. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (µg/ml)</td>
<td>1.18 (0.81, 2.54)</td>
<td>1.15 (0.55, 2.35)</td>
<td>1.21 (0.56, 2.45)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.08 (0.73, 1.33)</td>
<td>3.09 (0.73, 1.33)</td>
<td>3.04 (0.57, 1.40)</td>
</tr>
<tr>
<td>Adjusted Mean Differences</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>VACS Index Score</td>
<td>-0.06 (-0.45, 0.34)</td>
<td>-0.06 (-0.45, 0.34)</td>
<td>-0.06 (-0.45, 0.34)</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>4.79 (4.60, 16.29)</td>
<td>4.79 (4.60, 16.29)</td>
<td>4.27 (4.23, 15.27)</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>-2.38 (3.74, 1.10)</td>
<td>-2.38 (3.74, 1.10)</td>
<td>-2.38 (3.74, 1.10)</td>
</tr>
</tbody>
</table>

**Cardiovascular Risk Among People Accessing Differentiated HIV Care in South Africa**

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**Background:** Sub-Saharan Africa has a growing burden of cardiovascular disease (CVD) among people living with HIV. Differentiated antiretroviral therapy (ART) services are being scaled up but do not always integrate management of CVD comorbidities. Our objective was to (1) describe the burden of CVD risk within a randomized trial of community- and facility-based HIV care in South Africa (the DO ART Study), (2) evaluate if effective ART is associated with CVD risk, and (3) determine whether this association differs by mode of HIV service delivery.
Methods: We assessed CVD lifestyle risk factors (tobacco use, exercise, diet) and clinical measures (blood pressure, BMI, hemoglobin A1c, total cholesterol) at 12 months after ART initiation. To compare CVD risk by service delivery mode and viral suppression status, we estimated relative risks of hypertension, obesity/overweight, pre-diabetes/diabetes, hypercholesterolemia (defined using ACC/AHA thresholds), and tobacco use, and differences in underlying continuous measures, using generalized estimating equations for Poisson and linear regression, respectively. We assessed whether the relationship between viral suppression and CVD risk was modified by whether clients received community- or facility-based care.

Results: Among 1010 eligible participants, the median age was 32 years, 505 (50%) were men, 245 (24%) were current smokers, 468 (46%) exercised ≤2 days/week, 280 (28%) rarely ate vegetables, 450 (45%) had elevated blood pressure including 229 (23%) with hypertension, 502 (50%) had BMI ≥25 including 183 (18%) with BMI ≥30, 62 (6%) had prediabetes or diabetes, and 12 (1%) had hypercholesterolemia. CVD risk did not significantly differ by mode of service delivery. Visually suppressed persons had on average 5.75 mg/dl higher cholesterol (p = 0.001), 0.95 kg/m2 higher BMI (p = 0.003), and 16% higher risk of being overweight (p = 0.03) compared with clients who were not visually suppressed. Associations between viral suppression and CVD risk were stronger for community-based care and null for facility-based care.

Conclusion: In a relatively young population in South Africa, clients accessing community- and facility-based HIV care had substantial burden of tobacco use, hypertension and overweight. Among clients accessing community-based care, CVD risk factors were more prevalent among visually suppressed participants at 12 months. As community-based ART is scaled up, programs should evaluate integrated CVD screening and treatment services.

Distribution of clinical measures of cardiovascular risk, by viral suppression status at endline [dashed lines are group medians]

667 GUT MICROBIOTA ASSOCIATED WITH OBSTRUCTIVE CORONARY ARTERY DISEASE IN HIV INFECTION
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COCOMO study
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Background: We aimed to assess the potential impact of gut microbiota alterations and related pathways, on the presence and severity of stable coronary artery disease (CAD) in people living with HIV (PLWH).

Methods: Gut microbiota profiles from participants of the Copenhagen Comorbidity in HIV infection (COCOMO) study were determined by 16S rRNA sequencing, and CAD severity was assessed by a high-resolution research coronary CT angiography. Obstructive CAD was defined as >50% stenosis and non-obstructive CAD as 1-49% stenosis. Sequencing was performed on the Illumina MiSeq platform. α-diversity, β-diversity and bacterial abundances were analyzed on a rarefied (subsampled) dataset, and differentially abundant bacterial genera were adjusted for False Discovery Rate (FDR) using the Benjamini-Hochberg method. Associations between CAD-related microbiota alterations and obstructive CAD were tested using regression models adjusted for age, sex, and smoking.

Results: In a total of 254 participants (mean age 53y, 88% men) with microbiota samples and CT angiography, n=60 had obstructive CAD, n=60 had non-obstructive CAD, and n=134 had no CAD. Participants with obstructive CAD had gut microbiota profiles with significantly lower α-diversity and higher β-diversity compared to both non-obstructive CAD and no CAD. Participants with obstructive CAD also had distinct compositional microbiota profiles, including increased relative abundance of Ruminococcus Gnavus, known to produce pro-inflammatory polysaccharides and Veillonella, potentially linked to fibrosis, as well as reduced abundance of several bacterial genera, some diagnosed HIV 21 years (IQR 15-28), all virally suppressed on antiretroviral therapy, 14 (39%) had not had SARS-CoV-2 infection, 12 (32%) had prior SARS-CoV-2 infection without PASC, and 11 (30%) had PASC (Long COVID symptoms at CPET). Median CD4 count was 608 (370-736) and CD4/CD8 ratio 0.92 (0.56-1.27). Peak V02 was reduced among PLWH compared to individuals without HIV with an achieved exercise capacity only 80% vs 99% (p=0.005, Fig.), a difference in peak VO2 of 5.5 ml/kg/min (95%CI 2.7-8.2, p< 0.001). Exercise capacity did not vary by SARS-CoV-2 infection among PLWH (p=0.48 for uninfected vs infected; p=0.23 for uninfected vs no PASC; p=0.12 no PASC vs PASC). Chronotropic incompetence was present in 38% of PLWH vs 11% without HIV (p=0.002), and AHRR (normal >80%) was significantly reduced among PLWH vs individuals without HIV (60% vs 83%, p< 0.0001, Fig.). Heart rate response varied by SARS-CoV-2 status among those with HIV: namely, 3/14 (21%) without SARS-CoV-2, 4/12 (25%) with SARS-CoV-2 without PASC, and 7/11 (64%) with PASC (p=0.04 PASC vs no PASC). Among PLWH, CD4 count, CD4/CD8 ratio, and hsCRP were not associated with peak VO2 or AHRR.

Conclusion: Exercise capacity is reduced among PLWH, with no differences by SARS-CoV-2 infection or PASC. Chronotropic incompetence may be a mechanism of reduced exercise capacity among PLWH.

Exercise Capacity and Chronotropic Response by HIV/SARS-CoV-2

666 EXERCISE CAPACITY IS REDUCED IN HIV INDEPENDENT OF SARS-COV-2 INFECTION
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Background: Reduced exercise capacity occurs as a post-acute sequela of COVID-19 (“PASC” or “Long COVID”). Cardiopulmonary exercise testing (CPET) is the gold standard for measuring exercise capacity and identifying reasons for exercise limitations. Only one prior study used CPET to examine exercise limitations among people living with HIV (PLWH). Extending our prior findings for exercise limitations. We performed CPET within a COVID recovery cohort that included PLWH with or without PASC with: (1) exercise capacity (peak oxygen consumption, V02) and (2) adjusted heart rate reserve (AHRR, marker of chronotropic incompetence) using linear regression with adjustment for age, sex, and body mass index.

Methods: We assessed CVD lifestyle risk factors (tobacco use, exercise, diet) and clinical measures (blood pressure, BMI, hemoglobin A1c, total cholesterol) at 12 months after ART initiation. To compare CVD risk by service delivery mode and viral suppression status, we estimated relative risks of hypertension, obesity/overweight, pre-diabetes/diabetes, hypercholesterolemia (defined using ACC/AHA thresholds), and tobacco use, and differences in underlying continuous measures, using generalized estimating equations for Poisson and linear regression, respectively. We assessed whether the relationship between viral suppression and CVD risk was modified by whether clients received community- or facility-based care.

Results: Among 1010 eligible participants, the median age was 32 years, 505 (50%) were men, 245 (24%) were current smokers, 468 (46%) exercised ≤2 days/week, 280 (28%) rarely ate vegetables, 450 (45%) had elevated blood pressure including 229 (23%) with hypertension, 502 (50%) had BMI ≥25 including 183 (18%) with BMI ≥30, 62 (6%) had prediabetes or diabetes, and 12 (1%) had hypercholesterolemia. CVD risk did not significantly differ by mode of service delivery. Visually suppressed persons had on average 5.75 mg/dl higher cholesterol (p = 0.001), 0.95 kg/m2 higher BMI (p = 0.003), and 16% higher risk of being overweight (p = 0.03) compared with clients who were not visually suppressed. Associations between viral suppression and CVD risk were stronger for community-based care and null for facility-based care.

Conclusion: In a relatively young population in South Africa, clients accessing community- and facility-based HIV care had substantial burden of tobacco use, hypertension and overweight. Among clients accessing community-based care, CVD risk factors were more prevalent among visually suppressed participants at 12 months. As community-based ART is scaled up, programs should evaluate integrated CVD screening and treatment services.

Distribution of clinical measures of cardiovascular risk, by viral suppression status at endline [dashed lines are group medians]
with potential for butyrate production, important for gut barrier integrity. These bacterial genera (all \(Q_{FDR}<0.05\)) were used to define a CAD-related microbiota index: Loge (Prevotella_9 + Megapathera + Morrella + Catenibacterium + Fusibacterium + Ruminococcus_UG_005 + Ruminococcus_UG_009 + Lachnospiraceae_ND3007_group + Eubacterium_xylanophilum_group), which was associated with obstructive CAD independent of age, sex and smoking (adjusted \(p<0.001\)). We observed no differences in gut microbiota composition between PLWH with non-obstructive CAD and no CAD.

**Conclusion:** PLWH with obstructive CAD have distinct gut microbiota profiles compared to PLWH with non-obstructive CAD and no CAD. Future studies from this longitudinal cohort will determine whether these CAD-related microbiota profiles are predictive of future cardiovascular events in PLWH.

**Microbiota alterations associated with obstructive CAD**

![Image]

**PLAQUE IN WIHS WOMEN**

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*Presented at CROI by a nonauthor colleague*

**Background:** Microbiota alterations associated with obstructive CAD were inversely associated with and the other plaque-related metabolites (IPA) (OR = 0.62, 95% CI: 0.4, 0.98) and IPA/KYNA (OR = 0.51, 95% CI: 0.33, 0.8). We identified six gut bacterial genera associated with IPA levels (FDR-q < 0.25), five of which and multiple species within them were positively with IPA, most especially Roseburia sp, Eubacterium sp, Lachnospira sp, and Coprobacter sp. No gut bacteria were associated with KYNA levels. Furthermore, a constructed IPA-associated-bacteria score was inversely associated with plaque (OR = 0.47, P = 0.004), and the association was attenuated after further adjustment for IPA levels (OR = 0.54, P = 0.02) or IPA/KYNA (OR = 0.58, P = 0.05). No effect modification by HIV serostatus was observed.

**Conclusion:** In a subset of WIHS women, plasma IPA levels and related gut bacteria were inversely associated with carotid artery plaque, suggesting a potential beneficial role of IPA and its gut bacterial producers in atherosclerosis and CVD.

669 **MULTI-OMICS ANALYSIS OF GUT MICROBIOME AND ATHEROSCLEROSIS IN WOMEN LIVING WITH HIV**

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**Background:** Microbiota alterations associated with carotid artery plaque in up to 433 WIHS women. In a subset of WIHS women, plasma IPA levels and related gut bacteria were inversely associated with carotid artery plaque, suggesting a potential beneficial role of IPA and its gut bacterial producers in atherosclerosis and CVD.

668 **TRYPTOPHAN METABOLISM, THE GUT MICROBIOME, AND CAROTID ARTERY PLAQUE IN WIHS WOMEN**

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*Presented at CROI by a nonauthor colleague*

**Background:** The perturbation of tryptophan (TRP)-kynurenine (KYNA) metabolism has been linked with HIV infection and cardiovascular disease (CVD), but the relationship of other TRP metabolites with the gut microbiome and atherosclerosis is not fully understood within the context of HIV infection.

**Methods:** Ten TRP metabolites, including microbial indole derivatives, were quantified in plasma among women from 3 sites of the Women’s Interagency HIV Study between 2018-2019. The gut microbiome was profiled through 16S ribosomal RNA sequencing and shotgun metagenomics in fecal samples of HIV (65% HIV+) in the Women’s Interagency HIV Study (WIHS). We further integrated plaque-associated microbial features with serum proteomics (74 inflammatory markers measured by the proximity extension assay) and plasma metabolomics (378 metabolites measured by liquid chromatography tandem mass spectrometry) in relation to carotid artery plaque in up to 433 WIHS women.

**Results:** Fusobacterium nucleatum, a potentially pathogenic bacteria, was positively associated with carotid artery plaque, while five microbial species (Roseburia hominis, Roseburia inulinivorans, Johnsonella ignava, Odontobacter splanchinicus, Clostridium saccharolyticum) were inversely associated with plaque (Fig 1A). Results were consistent in both women with and without HIV. F.nucleatum was positively associated with and the other plaque-related species were inversely associated with multiple serum proteomic inflammatory markers, such as pro-inflammatory chemokines CCL5 and CCL1, which were also positively associated with plaque (Fig 1B, C). Associations between bacterial species (especially F.nucleatum) and plaque were attenuated after further...
adjustment for proteomic inflammatory markers. Plaque-associated species were correlated with several plasma metabolites (Fig 1C), including a microbial metabolite, imidazole-propionate (ImP), which was positively associated with plaque (P trend = 0.043) and several pro-inflammatory markers (eg. CXCL9 and CX3CL1, all P < 0.001). Further analysis identified additional bacterial species and bacterial hutfi gene (encoding enzyme histidine ammonia-lyase in ImP production) associated with plasma ImP levels.

Conclusion: Among women living with or at risk of HIV, we identified several gut bacterial species and a microbial metabolite ImP associated with carotid artery atherosclerosis, which might be related to host immune activation and inflammation.

Figure 1. Associations among gut microbiota, circulating proteomic inflammatory markers and metabolites, and carotid artery atherosclerosis.

670 IMPROVED GUT MICROBIOTA RECOVERY IN LATE HIV-1 PRESENTERS INITIATING DTG VS DRV/r
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Background: Late HIV-1 presenters have severely impaired systemic and intestinal immune function, increased immune activation, inflammation and gut dysbiosis, reflected by loss of microbial richness and diversity alongside disbalances in commensal bacterial species. Whereas immune and inflammation parameters gradually, albeit partially, recover following antiretroviral treatment (ART) initiation, it is unknown if (a) ART initiation is also faster than others. Here, we aimed to answer the latter two questions.

Methods: This was a substudy of ADVANZ-4 (NCT02337322), a multicentre, open-label, 2-arm randomized clinical trial, where ART-naïve subjects with ART-naïve subjects with HIV-1 and <100 CD4+ T-cells/mm³ were allocated 1:1 to initiate dolutegravir (DTG) or darunavir (DRV)/cobicistat (Cobicistat)/FTC/TDF (the control arm). After 24 weeks, 48 and 96 weeks, some groups were switched to dolutegravir and others to darunavir.

Results: 104 patients in the parent trial received their allocated intervention. Of these, 88 subjects (46 DTG and 42 DRV/r) provided fecal samples at 2 or more time points or at least 1 sample at week 0 and were included in this substudy. Their median CD4+ counts at baseline were 33 (13–67) cells/mm³. Study groups were well-balanced and with no significant differences in the microbiome at baseline. Gene richness increased overall, but increases were greater and only statistically significant in the DTG group (Table). In addition, B. adolescents, B. longum and U. scatoligenes became significantly more abundant in the DTG than DRV/r group at weeks 24, 48 and 96. Increases in microbial diversity were nominally larger in subjects increasing their CD4+ > counts by more than >150 cells by week 96 (LMM ANOVA, p = 0.082).

Conclusion: ART initiation in very advanced late presenters is associated with improvements in gut microbiome markers of better overall health during the first two years. Such improvements are greater if subjects initiate dolutegravir than darunavir/r, and likely mirror immune recovery kinetics.

Microbiome marker changes per group at baseline and week 96
Table 1: Outcomes from switch to TDF/3TC/DTG by original treatment received: CHARACtERISE trial

<table>
<thead>
<tr>
<th>Group</th>
<th>TAF/FTC/DTG</th>
<th>TDF/3TC/DTG</th>
<th>TDF/PCFT/DTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>74.6 (64.8-84.8)</td>
<td>74.6 (66.2-86.4)</td>
<td>74.6 (64.8-84.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 (22.3-26.3)</td>
<td>24.3 (22.3-26.3)</td>
<td>24.3 (22.3-26.3)</td>
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</tbody>
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672 FAVORABLE METABOLIC OUTCOMES 48 WEEKS AFTER SWITCH TO DTG/3TC
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Background: Since 1996, triple drug antiretroviral therapy (ART) is standard in HIV care. Nowadays, with the increased viral potency of new antiretroviral drugs, dual ART has become a valid and potentially less toxic alternative considering the (present) lifelong need of ART. Metabolic complications such as weight gain have been reported with newer agents such as integrase inhibitors and the nucleoside reverse transcriptase inhibitor tenofovir alafenamide (TAF). We report week 48 results of Rumba, the first open-label randomized clinical trial evaluating the effects on metabolic health of switch from 2nd generation integrase inhibitor based triple ART towards dual ART DTG/3TC.

Methods: Virosuppressed patients were randomized 1:2:1 to switch to DTG/3TC or to switch or stay on BIC/FTC/TAF. BMI, waist circumference as well as lipids and insulin resistance (HOMA-IR) were compared among both groups. Body composition and fat distribution were measured by dual-energy x-ray absorptiometry (DXA) scans; liver fibrosis by fibroscan. Primary endpoint analysis on the viral reservoir will be discussed separately. Inflammatory cytokines were measured using commercially available ELISA and Lumex kits. Linear mixed models were built to evaluate changes over time between the groups over baseline, week 24 (if available) and week 48.

Results: From 134 randomized patients, 130 (N=87 DTG/3TC, N=43 BIC/FTC/TAF) were included in the intention-to-treat-exposed analysis. The majority is male (71.5%), mean age is 46.5±11.8 years. 102 patients (78.5%) had European and 14 (10.8%) had African ethnicity. Significant changes were observed between the groups from baseline to week 48 (estimated mean difference with 95%CI; BIC/FTC/TAF minus DTG/3TC): ALT (5.37 [0.38, 10.37] U/L; p=0.035), HDL cholesterol (-2.77 [-5.46, -0.08] mg/dL; p=0.044), lean trunk mass (-595.14 [-1121.82, -68.45] g, p=0.027) and fat percentage (1.37 [-4.34, 2.28] %; p=0.003). The estimated differences in trunk fat mass (-617.63 [-1684.75, 130.99] g; p=0.067) and lean body mass (-776.88 [-1684.75, 130.99] g, p=0.003) were not significant. We observed significant divergences in other lipid parameters including triglycerides, LDL and total cholesterol nor in glucose, insulin, HOMA-IR and liver fibrosis.

Conclusion: Our data suggest that treatment with DTG/3TC has a favorable impact on week 48 metabolic outcomes as compared to treatment with BIC/FTC/TAF. More longitudinal data (up to week 144) are being collected.

673 REVERSIBILITY OF TAF- AND/OR INSTI-ASSOCIATED WEIGHT GAIN
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Background: Since the introduction of reverse transcriptase inhibitors (RTI) and integrase strand transfer inhibitors (INSTI) into the antiretroviral (ARV) armamentarium, dual ART has become a valid and potentially less toxic alternative considering the lifelong need of ART. We report on weight change in people on tenofovir alafenamide (TAF), dual ART with or without INSTI, with or without weight gain (WG) related to TAF and/or INSTI.

Methods: From the Dutch ATHENA Cohort, we selected all PWH with ≥7% WG before starting and after stopping either TAF, INSTI or both; versus 800 PWH with ≥7% WG continuing TAF and/or INSTI.

Results: 800 PWH who continued TAF and/or INSTI after ≥7% WG (245 only TAF; 347 only INSTI; 208 TAF+INSTI), the adjusted mean weight change at 24 months after first recording of ≥7% WG was -0.77 kg [-1.32 to -0.21].

Conclusion: TAF and/or INSTI-associated WG of ≥7% appears to be only partly reversible after discontinuation of TAF and/or INSTI, with relatively modest improvement in BMI category. In contrast, in those continuing TAF and/or INSTI first recording of ≥7% WG, weight remains relatively unchanged. Characteristics of 69 PWH with ≥7% WG before starting and after stopping either TAF, INSTI or both; versus 800 PWH with ≥7% WG continuing TAF and/or INSTI.

674 WEIGHT GAIN AMONG PARTICIPANTS SWITCHING TO A DOLUTEGRAVIR-BASED HIV REGIMEN IN KENYA
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Background: Dolutegravir (DTG) use has been associated with increased risk for weight gain. We have previously demonstrated that ART-naïve patients starting DTG in Kenya gain significantly more weight compared to those starting a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI). In this study we determine the impact of switching from NNRTIs, efavirenz (EFV) or nevirapine (NVP), to DTG-containing ART on weight gain in a low-income east African country with high prevalence of HIV and recent large-scale roll-out of DTG.

Methods: Participants enrolled in the Kenyan Academic Model Providing Access to Healthcare program who had been on NNRTI for at least 24 months prior to switching to DTG were included in the analysis. We excluded participants who switched due to virologic failure, women who were pregnant within 2 years of switching, and participants with missing BMI data at time of switch. Weights within 18-month of switch were included in the analysis. Body mass index (BMI) and weight were measured at baseline, week 24 (if available) and week 48.

Results: 23,131 participants met our inclusion criteria with 52% females, 28% with BMI ≥25 kg/m², 71% switching from EFV, and 29% switching from NVP. At the time of the switch, the mean age was 51 ± 10 years, the mean CD4 count was 201 ± 165 cell/mm³, and the mean BMI was 23 ± 4 kg/m². Compared to males, females were older (52 vs. 49 years) and had higher BMI (24 ± 22) kg/m² at
the time of switch. Participants gained, on average, 1.36 ± 5.7 kgs during the entire study period at an average rate of 0.59 kg/year. The rate weight increase was significantly higher post-switch compared to pre-switch (0.79 vs. 0.44 kg/year, p < 0.0001) (1A). The rate of weight increase post-switch was higher for females compared to males (0.96 kg/year vs 0.62 kg/year, p < 0.0001) (1B), and for participants switching from EFV compared to NVP (1.12 kg/year vs. 0.002 kg/year, p < 0.0001) (1C).

**Conclusion:** In a large HIV cohort from east Africa, on stable NNRTI treatment, switching to DTG-based regimens was associated with a greater rate of weight gain compared to pre-switch. Despite having greater BMI at time of switch, females had greater weight gain post-switch compared to males. Weight gain was predominantly found in those switching from EFV, thus suggesting EFV is more weight suppressive than NVP.

Figure 1. Changes in weight over time among all HIV patients switching from NNRTI to DTG (A). Weight changes by sex and by baseline NNRTI are shown in (B) and (C) respectively.

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**675 RANDOMIZED CLINICAL TRIAL OF TRANSGENDER WOMEN SWITCHING TO BIC/F/TAF (MOBETTA TRIAL)**

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**Background:** Cardiometabolic disease in transgender women (TW) is affected by feminizing hormonal therapies (FHT), HIV and ART. We evaluated the tolerability of switching to bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) vs continued ART in TW.

**Methods:** 21 TW on FHT and suppressive ART were randomized 1:1 to switch to B/F/TAF (Arm A) or continue current ART (Arm B) and were followed for 48 weeks. Biomarkers and hormones were measured centrally; fasting glucose and lipids were measured in real time. DXA scan measured bone mineral density (BMD) and lean/fat mass, and FibroScan® hepatic fat (controlled continuation parameter, CAP). Wilcoxon rank-sum/pearson χ2 tests compared continuous/categorical variables (two-sided a=0.05).

**Results:** For TW (Arm A n=12, Arm B n=9) had median age 45 years; 95% were non-white, 70% were on elvitegravir- or dolutegravir-based ART with 57% TAF and 24% abacavir, 29% had hypertension, 5% diabetes and 62% dyslipidemia. Arm A/B had 91%/89% undetectable HIV-1 RNA at week 48 (w48). There were no adverse events. Arm B had more frequent moderate/severe hepatic steatosis (42% vs. 89%) and a greater decrease (-25 dB/m) in CAP score vs Arm A (-3 dB/m) at w48 (p=0.03). Baseline (BL) osteopenia (Arm A/B 42%/25%) and osteoporosis (17%/13%) were common. At wk 48, improvement in BMD category was more common in Arm A (36% vs 13%), and only 1 TW in Arm A worsened BMD category. BL lean/fat mass were similar. At w48, Arm A had stable lean mass but increased limb (3lbs) and trunk (3lbs) fat, with a decrease in android/gynoid fat ratio. Arm B had a slight decrease in limb (-0.4lbs) and trunk (~0.1lbs) fat. BL and w48 fasting lipid and glucose profiles were similar; however, HOMA-IR decreased in Arm A (2.6 to 1.8) and increased in Arm B (3.5 to 3.9). BL and w48 concentrations of oxidized LDL, adiponectin, sCD14, D-dimer, tissue factor, plasminogen activator inhibitor-1 (PAI-1), endothelin-1, extracellular newly-identified receptor for advanced glycation end-products (EN-RAGE), TNF receptors I/II were similar. Higher estradiol (r=0.45, p=0.04) correlated with higher EN-RAGE, and lower total testosterone (r=0.51, p=0.002) and sex hormone binding globulin (r=0.45, p=0.05) correlated with higher PAI-1 at BL.

**Conclusion:** In this cohort of TW, switch to B/F/TAF was safe and metabolically neutral. Despite greater fat gain on BIC/F/TAF, a trend toward improved insulin sensitivity was observed after switch. Further study is needed to better understand cardiometabolic disease burden in TW with HIV.

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**676 METABOLIC PROFILING SHOWS MORE LIPID ABNORMALITIES IN NNRTI COMPARED TO INSTI USERS**

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¹2000HIV

**Background:** Integrase strand transfer inhibitors (INSTI) are associated with weight gain, in contrast to the also commonly used non-nucleoside reverse transcriptase inhibitors (NNRTI). Plasma metabolic and lipoprotein profile of INSTI and NNRTI users were therefore compared.

**Methods:** The present study has a two-phase design and includes a discovery and validation cohort of INSTI and NNRTI users from the 2000HIV study (clinicaltrials NCT03994835) consisting of 1895 virally suppressed participants on long-term antiretroviral therapy (ART). The discovery and validation cohort comprised of 601 INSTI and 469 NNRTI users, and 327 INSTI and 232 NNRTI users, respectively. Untargeted plasma metabolomics profiling was performed using liquid chromatography–mass spectrometry (General Metabolites). Plasma lipoproteins composition was measured using nuclear magnetic resonance spectroscopy (Nightingale). Data on comorbidities and comedication were available for all participants.

**Results:** INSTI users were a bit younger (51 vs 53 years, P< 0.001), had a slightly higher BMI (25.2 vs 24.8, P=0.016) and shorter duration of ART (8 vs 11 years, P< 0.001). Most recent CD4+ T-cell count, history of cardiovascular disease and frequency of lipid lowering drugs use was not different. Untargeted metabolomics yielded 1,720 metabolites, of which 500 were identified as serum metabolites based on Human Metabolic Database library. Overall, we identified 81/500 (16.2%) significantly different metabolites with modest fold change (-1.2 to 0.7 log2FC) between INSTI and NNRTI users in the two cohorts (figure 1A). The majority (30.9%) of these belong to the class of lipids or lipid like molecules (figure 1B), with de novo fatty acid biosynthesis being the most downregulated. Furthermore, assessment of 132 lipoprotein related biomarkers showed decreased triglycerides and very low-density lipoproteins in INSTI users (figure 1C). lep is the most downregulated lipoprotein in INSTI users in previous studies, our results support more favorable lipid profiles in INSTI users than in NNRTI users.

**Figure 1A:** Differential expression metabolites of INSTI users compared to NNRTI users. Pathway analysis showed two upregulated and eight downregulated pathways in INSTI users (figure 1C), with de novo fatty acid biosynthesis being the most downregulated. Furthermore, assessment of 132 lipoprotein related biomarkers showed decreased triglycerides and very low-density lipoproteins in INSTI users (figure 1B). Compared to NNRTI users, INSTI users showed lower fatty acid biosynthesis and lower levels of atherogenic lipids. Despite observed weight gain in INSTI users in previous studies, our results support more favorable lipid profiles in INSTI users than in NNRTI users.

Figure 1A: Differential expression metabolites of INSTI users compared to NNRTI users. Fold change direction indicates change in INSTI users compared to NNRTI users. B: Significant up- and downregulated serum metabolites of INSTI users compared to NNRTI users with compound names and metabolic categories. C: Up- (brown) and downregulated (green) metabolic pathways in INSTI users compared to NNRTI users. D: Differentially expressed group representative lipoproteins in INSTI users compared to NNRTI users. All lipoproteins intercorrelated with representative lipoproteins displayed on the right.
**677 INSULIN RESISTANCE, DM 2, AND POTENTIAL IMPACT OF FIRST ART IN NAIVE HIV-SUBJECTS**

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**Background:** Metabolic alterations related to insulin resistance (IR), development of DM2 and hepatic steatosis (HS) are aspects of great interest in PLWH. There is little knowledge about the metabolic characteristics at the time of diagnosis of HIV infection in individuals who will develop DM2 during follow-up and the impact of initial ART on the evolution of these alterations.

**Methods:** 6007 patients included in prospective cohort (CoRIS) who initiated ART between Jan-2010 and Nov-2019, without HBV/HCV coinfection. All DM2 diagnoses during 5 years follow-up were collected and their characteristics were analyzed. In addition, a subanalysis of subjects who initiated and maintained the same ART for at least 24 months, between 01-2010 and 12-2019, was performed. Changes in TyG-insulin resistance and TyG-hepatic steatosis indexes were analyzed according to the four most frequent ART regimens used: TDF+3TC or FTC+EFV (N=638), TDF+RPV (N=521), TDF+DRV (N=211), ABC+DTG (N=600). Changes in TyG-insulin resistance and TyG-hepatic steatosis indexes were analyzed at 12 and 24 months after initiation of ART, only in patients who received TDF+RPV the proportion of cases with IR/hepatic steatosis was reduced, this difference being significant (Figure). A multivariate analysis was performed on factors associated with the presence of IR/hepatic steatosis by TyG indexes at 12 and 24 months, with TDF+RPV being associated with less steatosis, OR: 0.38 and 0.56 p< 0.05 respectively.

**Conclusion:** PLWH who develop DM2 have very high prevalence of insulin resistance prior to ART initiation. Patients with recently diagnosed HIV infection in whom metabolic alterations related to insulin resistance are determined should be closely monitored and appropriate measures should be implemented to reduce its impact. First ART regimen could condition a different evolution of metabolic alterations related to IR/hepatic steatosis. The TDF+RPV regimen has an excellent metabolic profile and could have a protective effect on IR/liver steatosis, as measured by noninvasive markers.

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**678 MITOCHONDRIAL HAPLOGROUPS, ANTIRETROVIRAL DRUGS, AND DIABETES RISK AMONG MEN WITH HIV**

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**Background:** Mitochondrial genetic variability is associated with diabetes mellitus (DM) risk among people with HIV (PWfH). However, it is unclear to what extent this association is driven by antiretroviral (ART) exposure.

**Methods:** We included men without DM who had fasting glucose (FG) data available at baseline from the Multicenter AIDS Cohort Study (MACS). DM was defined by fasting glucose ≥ 126 mg/dL, the use of DM medication, a DM diagnosis, or hemoglobin A1c ≥ 6.5%. Self-reported time-varying exposure to mitochondrial-toxic ART (D-drug: stavudine, zalcitabine, and didanosine) were obtained at 6-monthly visits. Haplogroups were inferred from genotyping data using HaploGrep. We used multivariable Cox regression to examine the race-stratified association between common European (UK, JT) or African haplogroups (L2, L3) and hazard of DM over time. We further examine the association with and without D-drug exposure, as well as with a statistical interaction term. Models were controlled for principal components of genetic ancestry, age, BMI, GHC or HBV infection, and smoking (never, ever, vs. current).

**Results:** Of 2598 men (1249 PWH and 1349 people without HIV [PWoh]), 667 were Black, 1616 were White, and median age at baseline was 44 years [IQR: 37, 50]. Among PWH of African origin, Haplotype L3 was associated with increased hazard of incident DM (HR: 1.92, 95% CI 1.2, 3.1), independent of covariates and D-drug exposures. D-drug exposure was independently associated with incident DM (HR: 2.8, 95% CI 1.5, 5.3). Men with haplogroup L3 and exposure to D-drugs took the shortest time to develop DM (median: 3.5 years), while men without L3 and D-drug exposures took the longest time to develop DM (median: 7 years; Figure 1A). No association between European haplogroup (UK or JT) and DM risk was observed. However, PWH with European haplogroup UK and exposure to D-drug had a significantly shorter time to incident DM, compared to other groups (Figure 1B). Mitochondrial haplogroups were not associated with incident DM among PWoh.

**Conclusion:** A common mitochondrial haplogroup L3 increases the risk of incident DM in African-ancestry men with HIV. This association was accentuated by exposure to mitochondrial-toxic ART, but was seen in all men having this haplogroup.
ASSOCIATION OF SEX HORMONES WITH INCIDENT DIABETES IN WOMEN WITH AND WITHOUT HIV

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Background: Higher levels of sex hormone binding globulin (SHBG) are associated with decreased risk for type 2 diabetes mellitus (T2DM) in men and women in the general US population. By contrast, higher total testosterone (TT) has been associated with increased T2DM in women, but not in men. Women with HIV (WWH) are reported to have lower androgen hormone levels but higher SHBG levels than women without HIV (WWOH). Few have examined the association of androgen hormones with incident T2DM in WWH. We evaluated the association of TT, dehydroepiandrosterone sulfate (DHEAS), a marker for adrenal androgen secretion, and SHBG with incident T2DM in WWH and WWOH.

Methods: As part of the sex steroid sub-study of the Women’s Interagency HIV Study (WIHS) beginning in April 2003, women had TT, DHEAS, and SHBG measured from morning blood draws. After exclusion of women with prevalent T2DM or who were pregnant, 929 WWH and 364 WWOH were included in the analysis and followed through 2019. Incident T2DM was defined by either report of anti-diabetic medication or by confirmation of a HgbA1c > 6.5%.

Contact: Rebecca A. Abelman, rebecca.abelman@ucsf.edu

Results: Nearly half of the women identified as Black. At the index visit, WWH were older (median 41 vs 36 yrs), less likely to be pre-menopausal (47% vs 65%) and had lower median BMI (27 vs 29 kg/m²). Among WWH, median CD4 count was 415 cells/ml and 70% reported taking antiretroviral therapy. WWH had 9389 person-years of follow-up and 137 (1.5%) developed T2DM; WWOH had 4009 person-years of follow-up and 50 (1.4%) developed T2DM. The table shows that after adjustment, higher DHEAS and SHBG were non-significantly associated with longer time to T2DM whereas higher TT was non-significantly associated with shorter time to T2DM regardless of HIV status. In WWH and WWOH, BMI, HCV status, and HIV-related factors were adjusted for current smoking, BMI, HCV status, and HIV-related factors.

Conclusion: We found that the association of TT, DHEAS, and SHBG with T2DM was in the expected direction regardless of HIV status, but associations were not significant. Whether the magnitude of these associations is altered in post-menopausal women needs study.

Relative Time to T2DM Among Women With and Without HIV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Testosterone</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>DHEAS (per log)</td>
<td>0.03</td>
<td>1.13</td>
</tr>
<tr>
<td>SHBG (per log)</td>
<td>0.28</td>
<td>0.08</td>
</tr>
</tbody>
</table>

IMMUNE CHECKPOINTS AND PANCREATIC BETA CELL DYSFUNCTION IN HIV

Luke Pryke, Ziyue Liu, Alka Khaitan, Emily Sims, Samir Gupta

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Background: HIV is comorbid with other chronic medical conditions. We have previously shown that HOMA-B as a measure of pancreatic beta cell function is impaired in ART-treated people with HIV (PW) compared to untreated PW and to people without HIV (PW0H). Furthermore, proinsulin to C-peptide ratio (PIC), a marker of beta cell stress, is surprisingly lower in PW not on ART and with lower CD4 counts, compared to those on ART and to PW0H, thereby suggesting that lack of ART and more impaired T cell function is associated with less beta cell injury. We hypothesized that the immune dysregulation, specifically heightened immune checkpoints (IC), found in untreated HIV is protective against beta cell impairment, similar to Type 1 DM and IC inhibitor-induced DM. Our objectives were to determine the relationships between IC and both HOMA-B and PIC in HIV.

Methods: We utilized a sample of 105 patients from four groups (39 PW0H, 15 ART naive PW with CD4 count < 350 cells/µl, 28 ART naive PW with CD4 count ≥ 350 cells/µl, 23 PW receiving suppressive ART). Soluble IC levels were measured using a magnetic bead-based multiplex assay (Human ProcartaPlex™ panel). We used ANOVA to compare mean soluble IC (PD-1, TIM-3, CTLA-4, CD72 and CD40) between each PW group and the PW0H group. We calculated Pearson correlations to assess the relationships between IC levels, HOMA-B, and PIC ratio controlling for age, race, sex and BMI.

Results: As shown in the Table, PW had higher circulating levels of immune checkpoints PD-1, CD72 and CD40 (p = 0.001). PW receiving ART had higher TIM-3 (p = 0.037). In the entire study population, we found that PD-1, TIM-3, and CD40 were inversely correlated with PIC ratio (each p < 0.05) and that TIM-3 was positively correlated with HOMA-B (p = 0.02). Stronger correlations were found in the ART-treated PW group between TIM-3 and PIC (p = 0.048) and between CTLA-4 and HOMA-B (p = 0.015).

Conclusion: In the entire study population, soluble TIM-3 was correlated with better pancreatic beta cell function and inversely correlated with beta cell stress. PD-1, CTLA-4 and CD40 were found to correlate with both HOMA-B and PIC, but not both markers, making their role in beta cell injury or protection inconsistent. These data suggest that the soluble IC TIM-3 may play a role in the preservation of pancreatic beta cell function in HIV. How these data might reflect effects of cellular bound TIM-3 remains unknown. These results should be confirmed in longitudinal studies of patients initiating ART.

Soluble serum immune checkpoints and markers of pancreatic function

Table 1. Soluble immune checkpoints with markers of pancreatic function and diabetes, comparing patients with and without HIV infection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HOMA-B</th>
<th>PD-1</th>
<th>TIM-3</th>
<th>CD40</th>
<th>CD72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pic</td>
<td>-0.08</td>
<td>-0.23</td>
<td>-0.09</td>
<td>-0.22</td>
<td>-0.22</td>
</tr>
<tr>
<td>PIC</td>
<td>-0.38</td>
<td>-0.21</td>
<td>-0.02</td>
<td>-0.18</td>
<td>-0.22</td>
</tr>
<tr>
<td>HOMA-B</td>
<td>-0.50</td>
<td>-0.15</td>
<td>-0.26</td>
<td>-0.24</td>
<td>-0.24</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>-0.00</td>
<td>-0.00</td>
<td>-0.10</td>
<td>-0.10</td>
<td>-0.00</td>
</tr>
<tr>
<td>CD40</td>
<td>-0.11</td>
<td>-0.02</td>
<td>-0.10</td>
<td>-0.10</td>
<td>-0.00</td>
</tr>
<tr>
<td>CD72</td>
<td>-0.09</td>
<td>-0.16</td>
<td>-0.16</td>
<td>-0.16</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

* p < 0.01; ** p < 0.05; *** p < 0.001

DISCREPANCY BUT CGM HAS NOT BEEN USED IN PLWH. WE AIMED TO INVESTIGATE THE AGREEMENT BETWEEN HBA1C AND INTERSTITIAL GLUCOSE IN PEOPLE LIVING WITH HIV

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Background: Diabetes is reported to be more common in people living with HIV (PLWH). There is no agreed diagnostic test for diabetes in PLWH. Guidelines advise against HbA1c due to reports that it is falsely low in this group. Continuous glucose monitoring (CGM) is useful to explore the HbA1c-glucose discrepancy but it is currently being used in PLWH. We aimed to investigate the agreement between HBA1c and interstitial glucose using CGM, according to HIV serostatus in 2 cohorts: Lusaka, Zambia and Brighton, UK.
A RANDOMIZED CONTROLLED TRIAL OF BERBERINE EFFICACY ON METABOLIC SYNDROME PLUS HIV

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Background: Antiretroviral therapy has increased the life expectancy of people living with HIV (PLWH). Nevertheless, this increase is not free of comorbidities, and metabolic syndrome is among the most prevalent. Berberine is an alkaloid that has been shown to ameliorate metabolic syndrome components like glucose tolerance and insulin resistance, but it had not been tested in PLWH. Our aims were to evaluate the efficacy of berberine for improving clinical features, insulin resistance, metabolic profile, and inflammatory markers.

Methods: A randomized, double-blind, placebo-controlled trial was performed in adults living with HIV under virological suppression and with metabolic syndrome diagnosis who were assigned to receive either berberine 500 mg TID or placebo for 20 weeks. The primary outcomes were a composite of weight reduction, insulin resistance decrease, and lipid profile improvement.

Results: Forty-three participants were randomized (22 in the berberine group (BBR) and 21 in the placebo group (CTR)); at the end, 19 in the BBR and 17 in the CTR group completed the intervention period and were analyzed. At 20 weeks, the BBR group showed the following improvements: reduction in weight and body mass index (2.8 kg, p = 0.001; 0.99, p = 0.003, respectively), lower insulin resistance revealed by a TyG*BM decrease of 8 points (p = 0.01); additionally, a reduction in TNF-alpha of 1 pg/ml was found (p = 0.023), and a tendency toward benefit was shown in HOMA-IR and HOMA-%beta (decrease 0.59, p = 0.085; increase 3.5%, p = 0.065, respectively). Conversely, the CTR group had higher total cholesterol and c-LDL (increase 22 mg/dL, p = 0.005; increase 20 mg/dL, p = 0.024, respectively), and worse IL-6 concentration (increase 0.69 pg/ml, p = 0.018). Furthermore, an increase in HOMA-IR (increase 0.23, p = 0.041) was concurrent with a decrease in beta-cell function (HOMA-%beta decreased 1%, p = 0.041) and higher C-peptide levels (increase 174 pg/ml, p = 0.039). See Table 1. There were no serious adverse effects in both group.

Conclusion: In PLWH under virological suppression, berberine improves metabolic syndrome by reducing weight and body mass index, insulin resistance, and proinflammatory profile, among a tendency to increase beta-cell function, while the control group showed worsening in total cholesterol, c-LDL, IL-6 levels, insulin resistance, and beta-cell function, at the end of the follow-up. Further studies with more people and longer intervention periods need to be explored.

A RANDOMIZED CONTROLLED TRIAL OF BERBERINE EFFICACY ON METABOLIC SYNDROME PLUS HIV

Table 1. Cardiometabolic surrogate indexes for the evaluation of insulin resistance, and glycemia context among HIV.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A (n=22)</th>
<th>Group B (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.1 (±8.8)</td>
<td>52.7 (±8.4)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Diabetes complications</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Mean HbA1c (mmol/mol)</td>
<td>44.5 (±13.6)</td>
<td>44.5 (±13.6)</td>
</tr>
<tr>
<td>Mean sensor glucose (mmol/l)</td>
<td>7.2 (±0.7)</td>
<td>7.2 (±0.7)</td>
</tr>
</tbody>
</table>

HOMA-IR: Homeostatic Model Assessment insulin resistance; AA: African American; T2DM: type 2 diabetes mellitus; HDL: high density lipoprotein; BMI: body mass index; TyG*BMI: triglycerides multiplied by BMI.
VITAMIN C DYSREGULATION IN HIV: CROSS-SECTIONAL STUDY OF RENAL LEAK IN HIV+ WOMEN

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Background: Reduced plasma vitamin C (vitC) concentrations in chronic diseases may result from abnormal urinary excretion of vitC: a renal leak. While low plasma vitC concentrations may be due to inadequate diet, vitC renal leak indicates underlying pathophysiology. Relationships between HIV+ status, renal leak, and plasma vitC concentrations are unknown. We investigated the prevalence and factors associated with vitC renal leak in a cohort of women with and without HIV.

Methods: We conducted an outpatient cross-sectional convenience sampling study of 96 women (40 HIV+, 56 HIV-). Clinical and HIV-related history were obtained using structured questionnaires. To determine primary outcome of vitC renal leak prevalence, subjects fasted overnight, and matched urine and plasma samples collected the following morning. VitC was measured by HPLC with coulometric electrochemical detection. VitC renal leak was defined as presence of urinary vitC at fasting plasma concentrations below 43.2µM, the vitC Minimum Elimination Threshold (MET) in women. Exploratory outcomes assessed clinical parameters associated with vitC renal leak.

Results: Compared with HIV- controls, HIV+ cohort had significantly lower mean plasma vitamin C concentration (13.8µM vs 57.3µM, p< 0.001 Wilcoxon), and higher prevalence of vitamin C renal leak (75% vs 4.3%, OR54, p< 0.001 Fisher’s Exact). HIV-renal leak and HIV-plasma vitC concentrations are significant at 5% level following change-in-effect analyses that adjusted for non-HIV-related covariates with significant between-group differences (age, hypertension, hemoglobin A1c, eGFR, liver enzymes). Among complete-data subsets of the full cohort, renal leak was associated with older age, higher BMI, hypertension, obesity, lower eGFR, and use of HAART therapy (p<0.01 for all, adjusted for multiplicity). Renal leak was not associated with Race/ethnicity, C4 count, viral load, or duration of HIV diagnosis (p>0.05 for all).

Conclusion: HIV is associated with measures of vitC dysregulation: low vitC concentrations and high prevalence abnormal vitC urinary loss (renal leak). Older age, HAART therapy and comorbidities associated with metabolic and cardiovascular risk (obesity, hypertension, dyslipidemia) may be more relevant than chronic immune activation from HIV. Further research is needed to explore metabolic and cardiovascular implications of vitC dysregulation in HIV, and potential benefit of early supplementation.

685 RECOVERY OF BONE MASS IN WOMEN ON DEPO-PROVERA AND TDF-BASED ART SWITCHED TO B/F/TAF

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Background: We previously found that concomitant depot medroxyprogesterone acetate (DMPA-IM) contraceptive use resulted in a doubling of BMD loss in women living with HIV (WLWH) initiating tenofovir disoproxil fumarate (TDF) -containing ART in the BONE: CARE study. We sought to determine whether BMD would recover when these women switched to tenofovir alafenamide fumarate (TAF)-containing ART over a two-year period through a Phase IV open-label hybrid randomized and quasi experimental intervention study, the BONE: STAR study.

Methods: At the end of their 2-year follow-up in the BONE: CARE study, WLWH on TDF and DMPA-IM were randomized in a 1:1 ratio to either continue on a TDF based ART regimen or switch to B/F/TAF for 2 years. A third group of WLWH on TDF and using non-hormonal contraception were offered B/F/TAF. Dual energy x-ray absorptiometry was used to measure BMD (lumbar spine (LS), total hip (TH) and femoral neck (FN)) at enrollment and at 6-monthly intervals thereafter.

Results: We enrolled 344 virally suppressed women; 125 non-hormonal contraceptive users switching from TDF to B/F/TAF, and 219 DMPA-IM users continuing on TDF (108) or switching to TAF (111). Mean age was 31.3 years (SD 4.2) years. Both non-hormonal and DMPA groups who switched to B/F/TAF had significant improvement in mean % BMD post switch with no significant differences except at the LS; % differences in mean BMD (-0.011% (-0.020, -0.001)) p=0.029 at the LS, (0.254% (1.022, 0.515), p =0.516 at the TH, and (-0.277(-1.361, 0.806)), P=0.615 at the FN. However, DMPA users had lower mean BMD Z-scores at all time points. Additional research should focus on the impact of switching to B/F/TAF for 2 years.

Conclusion: WLWH receiving TDF-containing ART, switching to B/F/TAF was associated with significant improvement in mean % BMD underscoring the promising role of newer bone-sparing ART in minimizing comorbid risks among WLWH. However, compared to non-hormonal users, DMPA-IM users had lower BMD Z-scores at all time points. Additional research should focus on the impact of switching to B/F/TAF for 2 years among women in the BONE: STAR Study.
QUARTERLY VITAMIN D3 SUPPLEMENTATION TO MITIGATE TENOFOVIR-ASSOCIATED BONE LOSS

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1Yale School of Medicine, New Haven, CT, USA, 2Peking Union Medical College Hospital, Beijing, China (People's Republic), 3Beijing Ditan Hospital, Beijing, China (People's Republic), 4Beijing Youan Hospital, Beijing, China (People's Republic), 5Yale University, Beijing, China (People's Republic), 6Yale University, New Haven, CT, USA, 7Columbia University Medical Center, New York, NY, USA

Background: Initiation of antiretroviral therapy (ART) with tenofovir disoproxil fumarate (TDF)-containing regimens is associated with bone loss and fracture in persons with HIV (PWh). Vitamin D3 (VitD) and calcium supplementation attenuates declines in bone mineral density (BMD); however daily oral regimens increase pill burden and complexity.

Methods: We conducted a randomized, double-blind, placebo-controlled trial at three HIV care centers in Beijing, China. ART-naive adults were randomized to receive 180,000 IU VitD oral solution (equivalent of 2000 IU daily) or placebo with initiation of TDF/lamivudine/efavirenz and at the point-of-care every 12 weeks for 48 weeks. All participants received an educational pamphlet regarding dietary calcium. BMD assessments via dual-energy x-ray absorptiometry were assessed at baseline and 48 weeks; 25-hydroxyvitamin D (25OHD) and bone turnover marker levels were measured at baseline, 24, and 48 weeks.

Results: In total, 198/247 (80.1%) of randomized participants completed 48 weeks of follow up (VitD =96, placebo=102). The study groups were similar at baseline with a mean age of 31 years, 99% men, BMI of 22 kg/m2, HIV viral load of 4.41 mean log copies/mL, and 50% meeting criteria for vitamin D deficiency (25OHD < 20 ng/mL). At 48 weeks 96% of patients in both groups had achieved viral suppression (< 200 copies/mL). Mean 25OHD levels increased in the VitD group from 20.5±6.7 to 37.7±9.4 ng/mL over 48 weeks—with 80.5% of patients achieving vitamin D sufficiency (25OHD >30 ng/mL)—but remained unchanged in the placebo group (Figure 1A). Within both groups, BMD declined significantly from baseline to 48 weeks at all sites (p<0.001), however mean percent decrease in BMD did not differ significantly between treatment groups at the lumbar spine (p=0.5), total hip (p=0.19), and femoral neck (p=0.24) (Figure 1B). A total of 8 patients in the treatment group and 18 patients in the placebo group reported an adverse event, with no cases of hypercalcemia or nephrolithiasis, and similarly low rates of falls and fractures.

Conclusion: Quarterly administration of an oral vitamin D3 supplement—equivalent to 2000 IU daily was well tolerated in our study population but did not attenuate declines in BMD after initiation of TDF/lamivudine/efavirenz or decrease bone turnover markers in comparison to placebo. Potential reasons include insufficient vitamin D dose, lack of calcium co-administration, and limited sample size.

Figure 1. Change in measures from baseline to 48 weeks. A) Mean (95%CI) percent change in BMD from baseline to 48 weeks at the femoral neck, total hip and lumbar spine. B) Changes levels of 25OHD, parathyroid hormone (PTH), and markers of bone formation (Procollagen Type 1 N-terminal Peptide, P1NP, and bone resorption (serum cross-linked C-telopeptide of type I collagen, CTx) at baseline, 24 and 48 weeks.

IL-1β AND IL-10 ARE ASSOCIATED WITH FASTER LUNG FUNCTION DECLINE IN PEOPLE WITH HIV

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Background: People living with HIV (PLWH) have an increased risk of chronic lung diseases. We aimed to investigate if markers of inflammation and monocyte activation are associated with faster lung function decline in PLWH.

Methods: We included 655 PLWH from the Copenhagen Comorbidity in HIV Infection (COCOMO) Study. Eligible participants were ≥25 years, had two spirometric tests and a baseline plasma sample available, and no evidence of hepatitis B or C co-infection. Inflammatory markers (interleukin (IL)-1β, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17A, tumor-necrosis-factor-alpha (TNFα) and interferon-gamma (IFNγ)) were measured by Luminex immunoassays and soluble CD14 (sCD14) and sCD163 by the ELISA method. Using linear mixed models with age, sex, ethnicity, smoking status, and BMI as fixed effects, we investigated whether an elevated cytokine level (defined as above the 75th percentile) was associated with faster lung function decline in PLWH.

Results: The majority of PLWH were males (85.2%) with undetectable viral replication (95.3%). The median follow-up time was 2.4 years. We found a faster decline in the forced expiratory volume in one second (FEV1) in PLWH with elevated IL-1β, with an additional decline of 10.3 mL/year (95% CI:2.1-18.6), p=0.014 (Figure 1). Likewise, elevated IL-10 was associated with an additional FEV1 decline of 10.0 mL/year (95% CI:1.8-18.2), p=0.017. We found no interaction between smoking status and IL-1β on FEV1 decline (p-interaction = 0.688).

Conclusion: IL-1β and IL-10 were independently associated with faster lung function decline in well-treated PLWH, suggesting that persistent systemic inflammation may play a role in the pathogenesis of chronic lung diseases in PLWH.

Figure 1: Additional FEV1 decline (mL/year) in people living with HIV with elevated versus low concentration of the inflammatory markers listed on the Y-axis. Blue color indicates a statistically significant finding. Abbreviations: FEV1, forced expiratory volume in one second; TNFa, Tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; IL-2, interleukin-2; IL-4, interleukin-4; IL-6,
interleukin-6; IL-10, interleukin-10; IL-17A, interleukin-17A; IFNγ, interferon-
gamma; sCD14, soluble CD14; sCD163, soluble CD163; hs-CRP, high-sensitivity C-reactive protein.

688 ASSOCIATION OF ANEMIA ON SURVIVAL AMONG PEOPLE WITH HIV AFTER ART INITIATION
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Background: Anemia continues to be an independent predictor of mortality in the ART era, but few analyses have focused on this association. If we are to intervene on anemia, we need to understand it more fully. We estimate the association between anemia and anemia severity with mortality following the initiation of ART among PWH in North America.

Methods: Within the NA-ACCORD, median hemoglobin measurements each year between 01/01/2007-12/31/2016 were categorized into mild (11.0-11.9g/dL men, 11.0-11.9g/dL women), moderate (8.0–10.9g/dL regardless of sex) and severe (< 8.0g/dL regardless of sex) anemia. Discrete time-to-event analyses and complementary log-log link models estimated crude and adjusted mortality hazards ratios (aHR) and 95% confidence intervals for the association between anemia and anemia severity with mortality. Models were adjusted for race/ethnicity, HIV risk, year, age, hepatitis B and C virus, hypertension, diabetes, chronic kidney disease, non-AIDS defining cancer, clinical AIDS diagnosis, median viral load, median CD4 count, and ART use the year of hemoglobin observation. Subgroup analyses were conducted stratified by sex.

Results: Among 67,228 PWH contributing 320,261 annual median hemoglobin observations, 257,293 (80%) were not anemic, 44,041 (14%) had mild, 18,259 (6%) moderate, and 668 (0.2%) severe anemia. Among females 31% of median observations, 257,293 (80%) were not anemic, 44,041 (14%) had mild, 18,259 (6%) moderate, and 668 (0.2%) severe anemia. Among males 18% in male PWH. The results of this study add to a growing body of literature that suggest detectable viremia including LLV could have adverse clinical consequences. We confirm previously observed negative clinical consequences of delays in ART initiation and regimen type. In comparison with boosted PI, InSTIs and NNRTIs were protective, whereas unboosted PI use had a greater risk. Higher CD4 counts were protective.

Conclusion: The results of this study add to a growing body of literature that suggest detectable viremia including LLV could have adverse clinical consequences. We confirm previously observed negative clinical consequences of delays in ART initiation and lower CD4 counts. The association with race noted in 16% and HLV in 7.4%. We observed an association with LLV, HLV and SNAEs with a graded hazard by level of viremia, table 1. Other factors associated with SNAEs were recorded {107 [3.8%] malignancies, 158 [5.6%] cardiovascular events, 204 [7.25%] cases of chronic kidney disease and 21 [0.8%] cases of cirrhosis}. A third of the participants had one or more episodes of VF, LLV was noted in 16% and HLV in 7.4%. We observed an association with LLV, HLV, and SNAEs with a graded hazard by level of viremia, table 1. Other factors associated with SNAEs were older age, female gender, Caucasian race, delays in ART initiation and regimen type. In comparison with boosted PI, InSTIs and NNRTIs were protective, whereas unboosted PI use had a greater risk. Higher CD4 counts were protective.

Results: 2814 participants [94% male, 40% Caucasian, 43% African American] were followed for a median of 10.1 years [IQR 4.8 to 18.2]. A total of 490 [17.4%] SNAEs were recorded [107 [3.8%] malignancies, 158 [5.6%] cardiovascular events, 204 [7.25%] cases of chronic kidney disease and 21 [0.8%] cases of cirrhosis}. A third of the participants had one or more episodes of VF, LLV was noted in 16% and HLV in 7.4%. We observed an association with LLV, HLV, and SNAEs with a graded hazard by level of viremia, table 1. Other factors associated with SNAEs were older age, female gender, Caucasian race, delays in ART initiation and regimen type. In comparison with boosted PI, InSTIs and NNRTIs were protective, whereas unboosted PI use had a greater risk. Higher CD4 counts were protective.

Conclusion: The results of this study add to a growing body of literature that suggest detectable viremia including LLV could have adverse clinical consequences. We confirm previously observed negative clinical consequences of delays in ART initiation and lower CD4 counts. The association with race and gender and ART categories, need further evaluation.

Table 1: Factors associated with Serious Non-AIDS Events in the NHS

<table>
<thead>
<tr>
<th>Factor Category</th>
<th>RR (95% CI)</th>
<th>Adjusted RR (95% CI)</th>
</tr>
</thead>
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<td>Gender</td>
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<td>1.00 (0.99, 1.00)</td>
<td>1.00 (0.99, 1.00)</td>
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ANY LEVEL OF DETECTABLE VIREMIA IS ASSOCIATED WITH SERIOUS NON-AIDS EVENTS
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Background: A proportion of HIV-infected persons on antiretroviral therapy [ART] have detectable viral loads [VL] at low levels without ever meeting criteria for virologic failure [VF]. The clinical consequences of this low-level viremia [LLV] are not well understood. In this study we examined the association between detectable VL and serious non-AIDS events (SNAEs)

Methods: We used data from the US Military HIV Natural History Study (NHS). The NHS is comprised of military beneficiaries who are enrolled early in infection and have access to care and medications. NHS participants who initiated ART after 1996 were included if they had two or more VLs measured using an assay with a lower limit of detection of 50 copies/mL, ≥ 6 months after ART initiation. VLs were categorized as LLV [51-199 copies/mL], high level viremia [HLV- 200-999 copies/mL], VF [≥200 copies/mL on 2 or more successive determinations or a single VL>1000 copies/mL] and virologic suppression [VS=≤50 copies/mL on all determinations and blips]. SNAEs evaluated are footnoted on table 1. We examined the first occurrence of a SNAE from any category. Factors significant at a p < 0.05 in the univariate model were included in the multivariable Cox proportional hazards models. We adjusted for gender, race, time to ART start; Age, VL category, ART regimen and CD4 count were analyzed as time updated variables.

Results: 2814 participants [94% male, 40% Caucasian, 43% African American] were followed for a median of 10.1 years [IQR 4.8 to 18.2]. A total of 490 [17.4%] SNAEs were recorded [107 [3.8%] malignancies, 158 [5.6%] cardiovascular events, 204 [7.25%] cases of chronic kidney disease and 21 [0.8%] cases of cirrhosis}. A third of the participants had one or more episodes of VF, LLV was noted in 16% and HLV in 7.4%. We observed an association with LLV, HLV, and SNAEs with a graded hazard by level of viremia, table 1. Other factors associated with SNAEs were older age, female gender, Caucasian race, delays in ART initiation and regimen type. In comparison with boosted PI, InSTIs and NNRTIs were protective, whereas unboosted PI use had a greater risk. Higher CD4 counts were protective.

Conclusion: The results of this study add to a growing body of literature that suggest detectable viremia including LLV could have adverse clinical consequences. We confirm previously observed negative clinical consequences of delays in ART initiation and lower CD4 counts. The association with race and gender and ART categories, need further evaluation.
CD4/CD8 AT 2 YEARS OF ANTIRETROVIRAL THERAPY AND INCIDENCE OF SERIOUS NON-AIDS EVENTS

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Background: While a low CD4/CD8 ratio during HIV treatment correlates with immune activation, its predictive value for identifying people living with HIV (PWV) at increased risk of serious non-AIDS events (SNAEs) remains debated. We used data from the Spanish CoRIS cohort to assess whether the CD4/CD8 ratio at year 2 of antiretroviral therapy (ART) predicts the incidence of SNAEs in the following 5 years.

Methods: Eligible individuals were PWV with ART initiation up to 2014 (to allow for a 7-year follow-up) and HIV-RNA < 50 copies/mL at 2 years. Participants with a history of AIDS events or SNAEs were excluded. The predictor variable was achieving a CD4/CD8 ratio above the cutoff (0.25, 0.5, and 1.0) at year 2 of ART initiation. The primary outcome was the cumulative incidence of SNAEs (major adverse cardiovascular event, neoplasia, or death) during the subsequent 5 years. Follow-up started at year 2 after ART initiation and ended at the earliest of loss to follow-up, ART discontinuation, or administrative end of follow-up. We estimated the 5-year risk via a double-weighted pooled logistic regression to account for selection bias due to censoring. Covariates included age, sex, date of enrolment, risk group, education level, geographical origin, AIDS diagnosis, HIV-1 RNA, and CD4/CD8 at ART initiation. We computed survival curves introducing a time-varying intercept to allow the hazard to vary over time.

Results: We included 4625 participants. Median age was 37 years, 87% were male, median CD4/CD8 ratio at ART initiation was 0.29 (IQR 0.17, 0.46). At 2 years after ART initiation, 75%, 53%, and 16% of participants achieved a CD4/CD8 ratio >0.25, >0.5, and >1.0, respectively. 11% were censored due to loss to follow-up or ART discontinuation, and 4% had a SNAE during follow-up. Figure 1 shows the survival curves and the odds ratio for the event during follow-up. Participants who did not reach a CD4/CD8 ratio of 0.5 had a significantly increased risk of SNAEs, which was higher if CD4/CD8 < 0.25. Sensitivity analyses with additional adjustment for CD4+ at year 2 yielded similar results. Exploratory analyses for each 0.10 increase in CD4/CD8 showed that 0.50 was the highest ratio that maintained a statistically significant association with the incidence of SNAEs.

Conclusion: This study provides new evidence that a low CD4/CD8 ratio (< 0.5) after 2 years of ART is associated with an increased risk of SNAEs during the following 5 years. Future studies should address possible differences over the longer term.

Figure 1. Survival curves for each cutoff

SOLUBLE IMMUNEOREGULATORY PROTEINS PREDICTIVE FOR COMORBID EVENTS IN PEOPLE WITH HIV

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Background: People with HIV (PWH) experience an increased risk of morbidity, partly driven by chronic immune dysfunction despite effective antiretroviral drug therapy (ART). HIV infection is characterized by persistent inflammation that promotes the accumulation of activated/exhausted lymphocytes with reduced effector function. We previously identified that several soluble immunoregulatory proteins associated with diminished lymphocyte effector function are predictive of comorbid outcomes in PWH. Using a unique panel of soluble immunoregulatory proteins and a nested case-control study from the AIDS Clinical Trials Group ALLRT cohort, we aimed to identify additional predictors of comorbid events to improve biomarker delineation that may, in conjunction, contribute to driving disease progression.

Methods: Study participants were evaluated at one-year post-ART at viral suppression and immediately preceding a comorbid event. Cases experienced a myocardial infarction (MI)/stroke event or cancer development. Controls were matched for age (median 45 years), sex (84% male), pre-ART CD4+ T cell count (median 213 cells/mm³), ART regimen at 1 year, and parent study. A novel soluble immunoregulatory multiplex panel was developed and measured by Luminex. Conditional logistic regression analysis assessed biomarkers as predictors for comorbid events at each timepoint. Regression models were adjusted for CD4 counts at year 1 and pre-event; noteworthy associations used a threshold of an effect size (adjusted odds ratio) per 1 IQR >1.5. Support vector machine modeling with recursive feature elimination selection designed to preserve case-control dynamic was used to assess models and area under the curve of the receiver characteristic (AUC ROC) were used to measure model accuracy.

Results: Higher levels of APRIL, CD26, and Gal-1 were significantly associated with MI/stroke at year 1 and pre-event timepoints (Figure 1A). Higher levels of CD137, Gal-3, and Siglec-7 were significantly associated with cancer at both timepoints. Machine learning-based modeling showed an improvement in case classification accuracy with the inclusion of newly measured biomarkers in identifying PWH who at year 1 and pre-event would develop MI/stroke or malignancy (Figure 1B).

Conclusion: We provide expanded insight on immunoregulatory proteins that appear predictive of cardiovascular and cancer events in PWH initiating ART and these multiple pathways may synergize in driving disease.

Figure 1. Soluble immunoregulatory proteins in predicting myocardial infarction (MI)/stroke or malignancy at one-year post-ART initiation and pre-event. Conditional logistic regression models adjusted for CD4 counts (AJ), AOC <0.001, 95% CI 0.001-0.002, *P <0.05, **P <0.01, ***P <0.001. Support vector machine modeling with recursive feature elimination selection area under the curve (AUC) of receiver operating characteristic (ROC) to measure model accuracy. (B) Model parameters are listed (darker red connect) for each RLC, inclusion of newly measured biomarkers in bold. Blue line = ROC. Light grey area = standard deviation; red dotted line=chance.

TELOMERE LENGTH IN AVIREMIC PWH: HOW DIFFERENT IS IT FROM PERSONS WITHOUT HIV?

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Background: Blood telomere length (BTL) attrition is a surrogate biomarker of immunosenescence and aging associated with HIV infection. BTL recovery in long-term virologically suppressed PWH, but it is unknown if recovery is complete compared with persons without HIV.

Methods: Prospective 6-year observational study assessing the evolution of BTL in virologically suppressed PWH and a cross-sectional analysis comparing BTL with age and sex-matched blood donors, and sex matched persons older than 60 from a general population cohort. Relative BTL was determined by
monochrome quantitative multiplex PCR assay and expressed as the ratio of telomere to single-copy gene (T/S)

**Results:** We included 135 PLWH, 135 blood donors, and 135 persons over 60. Median age was 55 (IQR: 51-60), 55 (IQR: 51-60), and 70 (IQR: 68-73) years, respectively. 29.6% were women in each group. In the PWH group 43.7% and 34.8% were active/former smokers, 5.9% had hazardous alcohol consumption, 28.9% had been previously injected drugs users, 29.6% were Caucasian, 33.3% resolved hepatitis C virus infection, 91.1% positive CMV IgG and 70.4% with ≥ 1 comorbidity. At 6 years of follow-up, the median time with known HIV-1 infection and virological suppression was 23.1 (IQR: 18.4-27.6) and 13.4 (IQR: 12.5-14.0) years, respectively. Nadir/current median CD4 count were 180 (IQR: 71.5-258) and 780 (IQR: 535-1000) cells/μL, and the median CD4/CD8 ratio was 1.12 (IQR: 0.79-1.40). In the elderly population 9.6%, 45.2% and 45.2% were active, former and never smokers, respectively. BTL of PWH remained stable after 6 years of virological suppression (median BTL at baseline 1.03 (IQR: 0.92-1.14) vs. 1.03 (IQR: 0.94-1.15) after 6 years [p=NS]), without reaching the median BTL of blood donors of the same age and sex (1.25 (IQR: 1.11-1.45); p<0.001), but significantly above the median BTL of the older than 60 general population group (0.87 (IQR: 0.77-0.97); p<0.001) (Figure 1). 33 (24.4%) of PWH had BTL that was within ± 10% of the BTL of his/her age/sex matched blood donor. 27 (26.5%) of PWH < 60 had BTL that was within the ± 10% of the median BTL of the gender matched ≥ 60 participants from the general population.

**Conclusion:** During the 6 years of follow-up the median BTL of aviremic PWH remained stable but still was shorter than the BTL of age/sex matched blood donors. However, compared to the elderly population our data do not support that aviremic PWH have a very pronounced BTL shortening.

**Blood telomere length by group.*** ***p<0.001

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**693 BLOOD TELOMERE LENGTH GAIN IN PLWH SWITCHING TO DTG+3TC VS CONTINUING TRIPLE REGIMEN**

Francesca Lombardi, Alessia Sanfilippo, Massimiliano Fabbiani, Alberto Borghetti, Arturo Ciccoluto, Iolanda Mozzetta, Enrica Tamburrini, Simona Di Giambenedetto

**Background:** People living with HIV (PLWH) on long-term ART who maintain virological suppression continue experiencing blood telomere length (BTL) gain. However, ART containing NRTIs, such as tenofovir (TFV) or abacavir (ABC), which are potent inhibitors of human telomerase activity, has been shown to negatively impact the BTL increase. We investigated the effect on BTL at 1 year after switching to a dual therapy (DT) with dolutegravir (DTG) plus lamivudine (3TC) as the only NRTI vs maintaining a standard triple therapy (TT) with an anchor drug plus two NRTIs, one of which was TDF/TAF or ABC.

**Methods:** This was a prospective, longitudinal, matched, controlled study. We enrolled adults on stable (≥ 1 year) 3-drug ART and HIV-RNA< 50cps/mL who switched at baseline (BL) to DT or maintained TT. DT and TT groups were 1:1 matched for age, sex, years since HIV diagnosis, years on ART and anchor drug in the 3-drug regimen. BTL was assessed by a monochrome multiplex qPCR at BL and after 48 weeks (W48) and it was calculated as the telomere to albumin single copy gene (T/S) ratio. Comparison of BTL between and within groups was evaluated with parametric tests. Linear regressions were carried out to identify the variables associated with BL BTL and BTL changes over time.

**Results:** Between 2018-2021 we enrolled 120 PLWH, 60 in each group. The two groups were homogeneous for all main characteristics, except for slightly higher CD4 count in DT (Table 1). At BL, the BTL means (95%CI) were comparable between the two groups: 1.03 (0.98-1.08) for DT and 1.02 (0.96-1.07) for TT (p=0.704). At W48, viro-immunological status was stable and an overall increase in the mean (95%CI) BTL was observed, + 0.05 (0.02-0.08) (p=0.001). However, a within-group analysis showed a significant mean BTL gain in the DT group (+ 0.08 (0.04-0.12); p<0.001) but not in the TT group (+ 0.03 (-0.02-0.072); p=0.285). In a multivariable regression, younger age (p = 0.09 per +1 year increase; -0.011/-0.006; p<0.001), being female (vs male +0.098; 0.014/0.181; p=0.022) and higher CD4/CD8 ratio (+0.060; 0.0002/0.019; p=0.050) were independently associated with higher BL BTL. Higher BTL change was associated with DT group (+0.060; 0.003/0.017; p=0.041) and with shorter BL BTL (-0.027; -0.440/-0.153; p<0.001).

**Conclusion:** In this setting, switching to a dual regimen with 3TC plus DTG was associated with higher gains in BTL than maintaining triple therapy after the 1-year follow-up. These data suggest that dual therapy could have a positive effect on BTL.

**Table 1. Characteristics of participants at baseline**

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**694 COMPARISON OF TELOMERE LENGTH CHANGES OVER 48 WEEKS IN SALSA STUDY: DTG/3TC VS CAR**

Mark Underwood, James Oyee, Joseph Horton, Tatini Chakraborty, Chris Parry, Ruanal Wang, Yue Lin, Myoornan Sithamparanathan, Bryn Jones, Brian Wynnne, Choy Man, Andrew Zolopa

**Methods:** Adults with VL < 50c/ml for ≥6 months were randomized to DTG/3TC fixed-dose combination (FDC) or continued CAR with 81% (200/247) receiving TAF or TDF. qPCR using genomic DNA extracted from whole blood samples was performed to generate ratios of telomere TTAGGG repeats to a single-copy gene (T/S ratios) reflecting average TL of the total cell population. The adjusted mean difference (Figure 1): CD4+ cell count, age, sex, race group, depression or anxiety, BMI, smoking status, vitamin use, statin use, BL, TL time since first ART, and BL third agent class (PI, INSTI, or NNRTI).

**Results:** Adjusted mean changes from baseline to T/S ratio in the DTG/3TC and CAR groups were similar, -0.0020 vs -0.0048, respectively (treatment difference, 0.0028; 95% CI: -0.0146, 0.0202; P=0.701). Baseline factors associated with TL included age and sex (women had longer TL) and decreased TL is associated with disorders such as cardiovascular disease, stroke, and diabetes. For people living with HIV-1 (PWH) without treatment, TL shortening is seen, and has been associated with immune activation and lower immunological response. ARVs and regimen received can impact TL; tenofovir has been shown to inhibit telomerase activity in vitro, and in clinical studies may be associated with shorter TL. The SALSA study showed switching to DTG/3TC fixed-dose combination (FDC) was non-inferior to continuing current antiretroviral regimen (CAR) at Week 48 (respectively, 94% vs 93% with viral load (VL) < 50c/ml) by Snapshot analysis. This post-hoc analysis assesses TL from baseline (BL) through 48 weeks.

**Methods:** Adults with VL < 50c/ml for ≥6 months were randomized to DTG/3TC FDC or continued CAR with 81% (200/247) receiving TAF or TDF. qPCR using genomic DNA extracted from whole blood samples was performed to generate ratios of telomere TTAGGG repeats to a single-copy gene (T/S ratios) reflecting average TL of the total cell population. The adjusted mean difference in TL at Week 48 between treatment groups (DTG/3TC vs CAR) was calculated using an ANCOVA model including the following pre-specified covariates (Figure 1): CD4+ cell count, age, sex, race group, depression or anxiety, BMI, smoking status, vitamin use, statin use, BL, TL time since first ART, and BL third agent class (PI, INSTI, or NNRTI).

**Conclusion:** In the SALSA study, we show that age and sex significantly influenced TL as expected, however continuing TDF or TAF in the CAR arm did not appear to have an impact, though limitations include that 19% of participants in CAR arm did not receive a tenofovir produg. Of note, there was no impact on TL when switching to DTG/3TC over 48 weeks. This data, alongside non-inferior efficacy and minimal impact across inflammatory markers seen.
**695** SEVERAL InSTI AFFECT IMMUNE CELL MITOCHONDRIA, PROLIFERATION, AND APOPTOSIS Ex vivo

Renying Cai, Anthony Hisieh, Aya Zakaria, Helene C. F. Côté
University of British Columbia, Vancouver, BC, Canada

**Background:** HIV integrase strand transfer inhibitors (InSTIs) are popular among people living with HIV for their tolerability and low pill burden. However, compared to older antiretrovirals (ARVs), little is known about their mitochondrial toxicities. Dolutegravir has been associated with weight gain in adults, which may reflect changes in cellular metabolism regulated by mitochondria. Mitochondrial toxicity of more recently approved InSTIs bicitravir, elvitegravir+cobicistat, and cabotegravir remains unclear.

We herein characterized the effects of InSTI exposure on cultured immune cell mitochondrial health, and proliferation.

**Methods:** PBMCs from healthy volunteers were treated with T cell activator anti-CD3/CD28 for 6 days while also exposed to 1xCmax InSTIs in medium + 0.1% DMSO (ARV diluent). Mitochondrial intermembrane potential (MMP), reactive oxygen species (mtROS), and mass (mtmass), along with cellular proliferation and apoptosis, were determined by flow cytometry. Comparisons with the DMSO-exposed control cells were done by paired t-test. Additional experiments were performed to further explore changes in T-cell activation markers and T-cell memory compartments when exposed to InSTIs. These were carried out as above, with new antibodies for activation and differentiation.

**Results:** Compared to DMSO controls (n=9 biological replicates), bictegravir exposure exerted the most pronounced effects, with two-fold decreased mtmass, mtROS, MMP, a five-fold decrease in proliferation, and one-fold increase in apoptosis, while elvitegravir+cobicistat decreased MMP and mtmass, mtROS, MMP, a five-fold decrease in proliferation, and one-fold increase in apoptosis, were determined by flow cytometry. Comparisons with the DMSO-exposed control cells were done by paired t-test. Additional experiments were performed to further explore changes in T-cell activation markers and T-cell memory compartments when exposed to InSTIs. These were carried out as above, with new antibodies for activation and differentiation.

**Conclusion:** These data clearly show that some second generation InSTIs can affect mitochondria in cultured PBMCs. Furthermore, the effects of bictegravir ex vivo suggest a potential underlying metabolic mechanism which could hinder immune responses. Further exploration of the effect of InSTIs on T-cell activation is required as these types of toxicities may not be revealed by clinical trials yet could exert long-term immunological and health consequences.

**696** SARCOPENIA, FRAILTY, NUTRITION, AND HCV INFECTION AMONG OLDER PEOPLE WITH HIV

Hay Mar Su Lwin1, Win Min Han2, Tanakorn Apongpong2, Thanathip Wichiansan1, Jedsadakorn Bonrungsirisap1, Chanya Chatjitakornkul2, Matthew Sirisuksuksalcha2, Papitchaya Sukruen2, Sivaporn Gatechompol1, Stephen J. Kerr1, Anchalee Avihingsanon1

**Background:** Sarcopenia is a geriatric syndrome associated with a loss of muscle mass and functionality. It may increase morbidity and mortality. As Asian data are limited, we investigated the prevalence and risk factors for sarcopenia in older people living with HIV (PLWH).

**Methods:** We conducted a cross-sectional study in a cohort of virologically well-suppressed PLWH and age- and sex-matched HIV-negative controls, aged ≥50 years, from 2017 to 2018 in Bangkok, Thailand. Diagnosis of sarcopenia was based on Asian Working Group for Sarcopenia 2019 criteria: handgrip strength by a handheld dynamometer (male < 28kg, female < 18kg), walking speed 4-meter-walk < 0.8m/s and skeletal muscle mass (male < 7.0/kg/m²; female < 5.7/kg/m²) by bioelectrical impedance analysis. Osteoporosis was defined as T-score cutoff points ≤-2.5 at any site of DXA scan (lumbar spine, total hip and femoral neck). Frailty and nutritional status were evaluated using Fried’s criteria and Mini Nutritional Assessment, respectively. Multivariable regression analysis was used to assess the relationship between sarcopenia and participant demographic and clinical variables. We also compared bone turnover markers between those with or without sarcopenia.

**Results:** A total of 407 participants (277 PLWH and 130 controls) were included; 36% were female. Median age was 55 (interquartile range (IQR): 52-60) years and median duration of ART was 16 (IQR: 13-19) years. PLWH had a higher prevalence of sarcopenia (8.3% vs 3.1%; p=0.05), HCV infection (9.0% vs 2.3%; p<0.01), frailty (9% vs 3.1%; p=0.001) and at higher risk of malnutrition or malnourishment (18% vs 7%; p=0.002). Osteoporosis at any site was almost double in PLWH than controls (15% and 8.5%; p=0.06). Serum 25(OH)D, phosphorous, calcium, C-terminal telopeptide (CTX), amino-terminal pro-peptide of type-1 procollagen (P1NP) and intact Parathyroid Hormone (iPTH) were not significantly different between the sarcopenia groups. In a multivariate model among PLWH, BMI < 18.5kg/m², male sex, HCV coinfection, frailty and malnutrition were significantly associated with sarcopenia (Table).

**Conclusion:** In this aging cohort, PLWH had a higher burden of sarcopenia than HIV-negative individuals. Besides low BMI and male gender, hepatitis C co-infection, poorer nutritional status and frailty were identified as predictive risk factors. Therefore, interventions to improve nutritional status and early HCV treatment may reduce the risk of sarcopenia in older PLWH.

**Association between sarcopenia with sociodemographic and clinical-related factors among people living with HIV identified by multivariable logistic regression.**

**CMV IgG IS ASSOCIATED WITH MUSCLE FUNCTION BUT NOT QUALITY OR MASS IN PEOPLE WITH HIV**

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**REPRIEVE Study Team**

University of Colorado Anschutz Medical Campus, Aurora, CO, USA, *Harvard T.H. Chan School of Public Health, Boston, MA, USA, *University of Alabama at Birmingham, Birmingham, AL, USA, *Temple University, Philadelphia, PA, USA, *Massachusetts General Hospital, Boston, MA, USA, *University of Cincinnati, Cincinnati, OH, USA, *Ohio State University, Columbus, OH, USA, *Duke University, Durham, NC, USA, *The Johns Hopkins University, Baltimore, MD, USA

**Methods:** We investigated the association between CMV specific IgG and two measures of muscle function (CMYQ muscle quality and muscle mass) among individuals on ART treatment. Multivariable linear regression was used to test for associations for CMV titer with muscle quality and mass in an analysis of data from 933 ART-suppressed HIV-infected individuals from the REPRIEVE cohort. These analyses were adjusted for age, race, sex, BMI, alcohol intake, physical activity, and depression. Results: When stratified by CMV status and adjusting for potential confounders, higher titers of CMV IgG were associated with less muscle loss (p=0.03) among people with CMV IgG, but not among those without CMV IgG (p=0.68) (Table). These findings were consistent across subgroups of BMI, sex, and age. Conclusion: These findings are consistent with previous reports suggesting that CMV infection is associated with muscle function and may have potential clinical implications for interventions to reduce the effects of CMV infection on muscle mass and function.
Background: Cytomegalovirus (CMV) infection is associated with poor outcomes, including physical function impairment, in people without HIV. We examined associations of CMV IgG antibody titers with physical function and muscle quantity/quality in virologically suppressed middle-aged people with HIV (PWH), leveraging REPRIEVE baseline data.

Methods: REPRIEVE is a double-blind randomized trial evaluating pitavastatin for primary prevention of cardiovascular disease in PWH. This cross-sectional analysis focuses on participants enrolled in a substudy with additional biomarker testing and imaging at study entry. CMV IgG was measured in duplicate using the human CMV IgG enzyme immunoassay by Genway Biotech. Paraspinal muscle area (MA) and density (MD) were assessed on non-contrast CT. Physical function and frailty were obtained by Short Physical Performance Battery (SPPB), a modified version (mSPPB) and the Fried Frailty Phenotype, respectively. Associations between CMV IgG (risk factor) and outcomes were evaluated using partial Spearman correlation, and linear and log-binomial regression.

Results: Of 717 participants with CMV IgG measurements, the median age was 51 (Q1, Q3: 46, 56) years; 18% were natal female, 51% White, 37% Black, and 25% Hispanic; median BMI was 27 (24, 30) kg/m²; 93% had HIV-1 RNA <50 copies/mL, and 50% nadir CD4< 200 cells/mm³. Median CMV IgG was 27.6 (807, 6672) IU/mL, none below the limit of quantification. There was no evidence of association between CMV IgG and MA or MA density. The Mod-FP has good performance in measuring frailty among PWH and is reasonable to use when the gold standards of observed assessments (i.e., weakness and slowness) are not feasible.

Conclusion: The known association between CMV IgG and physical function or frailty is replicated in PWH and does not appear to be explained by changes in muscle quantity or quality, nadir CD4 or inflammation. Further mechanistic studies are needed to understand this association and whether CMV eradication or CMV-specific therapy can impact physical function in PWH.

Figure: Associations of CMV IgG with Physical Function

Table 1. Receiver operating characteristic (ROC) results for the classification of frailty and prefrailty by the Mod-FP compared to FFP.

<table>
<thead>
<tr>
<th>Mod-FP Stage</th>
<th>ROC AUC (95% CI)</th>
<th>Cut point (Mod-FP score)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frail</td>
<td>0.93 (0.85-1.00)</td>
<td>≥3</td>
<td>50%</td>
<td>15%</td>
</tr>
<tr>
<td>Prefrail</td>
<td>0.86 (0.82-0.90)</td>
<td>≥2</td>
<td>65%</td>
<td>68%</td>
</tr>
</tbody>
</table>

Abbreviations: AUC: area under curve, FFP: Fried Frailty Phenotype, Mod-FP: Modified Fried Phenotype, people with HIV

698 VALIDITY OF A SELF-REPORTED MODIFIED FRAILTY PHENOTYPE AMONG PEOPLE WITH HIV

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Background: Modifications to Fried’s frailty phenotype (FFP) are common, particularly to address data collection limitations in clinical care settings. We evaluated a self-reported modified frailty phenotype (Mod-FFP) used among people with HIV (PWH) within a US-based cohort and compared it to the original FFP.

Methods: Among 522 PWH who completed visits in the Impact of Physical Activity Routines and Dietary Intake on the Longitudinal Symptom Experience of people living with HIV (PROSPER-HIV) study within the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort, we assessed validity of the Mod-FFP using FFP as the gold standard. FFP includes unintentional weight loss, fatigue, and inactivity by self-report, and observed slow gait speed and weak grip strength. The Mod-FFP includes unintentional weight loss, fatigue, inactivity, and poor mobility all collected via self-report. Each component is assigned a point if present. FFP is scored 0-5 while Mod-FFP is scored 0-4. FFP categorizes frail as a score ≥3, prefrail as 1-2, and not frail as 0, and we identified cut points for Mod-FFP in this analysis. We compared the Mod-FFP with FFP via correlation, receiver operator characteristic (ROC) curves, agreement in classifying frailty status, and criterion validity via cross-sectional association with risk of having experienced falls.

Results: The median age of the cohort was 54 (IQR: 44-61), 21% were female, and 58% non-White. The Mod-FFP and FFP were highly correlated (Pearson r: 0.83), and the Mod-FFP classified 8% of PWH as frail, while FFP classified 9%. The area under the ROC curve for Mod-FFP classifying frailty was 0.93 (95%CI:0.91-0.96) with 62% sensitivity and 97% specificity at a cutoff of 3 components (Table 1). For prefrailty, at a cutoff of 1 component, the AUC was 0.86 (95%CI:0.83-0.89). ROC values were consistent in age (over/under 55), sex (male/female assigned at birth), and race (Black/White) stratification. We observed 80% agreement (unweighted kappa=0.64, quadratic weighted kappa=0.75) between the phenotypes for categorizing PWH as not frail, prefrail, or frail. Both phenotypes found frailty associated with falls; FFP (OR:1.63, 95%CI:1.22-2.18) estimated a greater magnitude for the association than Mod-FFP (OR:1.36, 95%CI:1.02-1.81), though the confidence intervals overlapped.

Conclusion: The Mod-FFP has good performance in measuring frailty among PWH and is reasonable to use when the gold standards of observed assessments (i.e., weakness and slowness) are not feasible.

Table 1. Receiver operator characteristic (ROC) results for the classification of frailty and prefrailty by the Mod-FFP compared to FFP.

<table>
<thead>
<tr>
<th>FFP Stage</th>
<th>ROC AUC (95% CI)</th>
<th>Cut point (Mod-FFP score)</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
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</table>

Abbreviations: AUC: area under curve, FFP: Fried Frailty Phenotype, Mod-FFP: Modified Fried Phenotype, people with HIV

699 COMORBIDITIES AND SYMPTOMS ASSOCIATED WITH FALLS: 2020-2021

Lydia Drumright1, Stephanie Ruderman1, Amanda Willig2, Bridget M. Whitney3, Robin Nance4, Andrew Hahn5, Sarah Mixson1, Jimmy Ma1, Rob Fredericksen2, Jeff Jacobson2, Katerina Christopoulous6, Sonia Napravnik1, Edward R. Cachay1, Allison Weibel1, Heidi Crane1

1University of Washington, Seattle, WA, USA, 2University of Alabama at Birmingham, Birmingham, AL, USA, 3Case Western Reserve University, Cleveland, OH, USA, 4University of California San Francisco, San Francisco, CA, USA, 5University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 6University of California San Diego, San Diego, CA, USA

Background: Falls are associated with aging-related decline in health, occurring more frequently among people 65 years-old and older. People with HIV (PWH) experience aging-related complications earlier than the general population. While falls have previously been associated with aging-related complications among PWH, this work is limited by small sample sizes, focused on only healthcare-reported falls, and often includes falls prior to the current antiretroviral treatment (ART) era.

Methods: We examined falls reported within the previous 12 months between 2020-2021 among PWH in the Center for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort at 7 US sites. Data were collected via patient reported and clinical measures. Associations of clinical, demographic and behavioral factors with any and number of falls were examined using relative risk regression models.

Results: Among 2386 PWH with complete data (mean age was 51 years and ranged 18-89 years; 14% were female and 53% non-white), 18% (n=435) reported falling at least once in the past year, among whom 50% fell once, 28% twice, and 22% ≥ 3 or more, with 27% (n=118) seeking medical care due to a fall. Falls were reported significantly more among PWH ≥ 50 years-old, however 9% of those under 40 years-old reported falling with half reporting more than one fall. Falls were also more likely to be reported by PWH who were white and lived near the West coast of the US. When adjusted for age, sex, location and race/ethnicity, falls were associated with reporting symptoms of...
neuropathy, forgetfulness, fatigue, feeling dizzy, and depression. A prefrail or frail phenotype, diabetes, lower quality of life (Qol) scores and more emergency visits were also associated with falls. Risk ratios generally increased with more falls (Table). However, HIV viral load and current and nadir CD4 count were not consistently associated with falls.

**Conclusion:** Almost 20% PWH in routine clinical care reported falling in the past year, including 100 PWH < 40 years-old. Neurological and mental health symptoms, frailty, diabetes, and lower Qol were strongly associated with both falls and number of falls. Our finding suggest that a high proportion of PWH, even younger people, experience falls, which may be indicative of aging-related issues. Monitoring of neurological, cognitive, and mental health symptoms across the age spectrum in routine clinical visits may be important among PWH to minimize complications with early onset aging.

Table: Factors associated with any and number of falls in the past 12 months adjusted for age, race/ethnicity (white vs all others), geographic location (west vs all others), and birth sex (N=2386)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Any Falls (n=2386)</th>
<th>1 Fall (n=2386)</th>
<th>2 or More (n=2386)</th>
<th>All (n=2386)</th>
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<tr>
<td>Demographics</td>
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<td>Age (years)</td>
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<td>Sex (male vs female)</td>
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<td>Race/Ethnicity (white vs others)</td>
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<td>Geographic location (west vs all)</td>
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<td>Medical history</td>
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<td>Diabetes</td>
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<td>Heart failure</td>
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<td>Mood disorder</td>
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<td>Frail phenotype</td>
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<td>Neurological symptoms</td>
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<td>Mental health symptoms</td>
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<td>Substance abuse</td>
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<td>Suicide attempt</td>
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<td>Exposure to illicit drugs</td>
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<td>Physical activity</td>
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**SMOKING CESSATION AND ASSOCIATED FACTORS IN THE HIV OUTPATIENT STUDY: 2007-2021**

Jun Li1, Carl Armon2, Alexander Ewing3, Jonathan Mahnen4, Ellen Tedaldi5, Frank Palella5, Richard Novak5, Cynthia Finhaver6, Stockton Mayer7, Andrea Wendrow8, Gina Simoncini9, Linda Battalora10, Kimberly Carlson11, Marcus Durham1, Kate Buchacz8

HIV Outpatient Study (HOPS) Investigators

1 Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Cerner Corp, Kansas City, MO, USA, 3University of Kansas, Kansas City, MO, USA, 4Temple University, Philadelphia, PA, USA, 5Northwestern University, Chicago, IL, USA, 6University of Illinois Chicago, Chicago, IL, USA, 7University Health, Denver, CO, USA, 8AIDS Healthcare Foundation, Philadelphia, PA, USA, 9Colorado School of Mines, Golden, CO, USA

**Background:** Smoking rates and cessation efforts vary by state, and the effectiveness of smoking cessation interventions among HIV patients is not well understood.

**Methods:** We analyzed data from the HOPS study, a multi-center, prospective, cohort study of HIV-infected adults initiated in 2007. The study included adults aged 18 years or older who presented for care at one of 10 sites across the United States. The primary outcome was smoking cessation at 6 and 12 months post-enrollment.

**Results:** Among the 1001 participants included in the study, 65% were male, 71% were black, and 74% were white. The median age at enrollment was 43 years (IQR 34-53). At baseline, 87% of participants were current smokers, and 13% were former smokers. Over the 6 and 12 month follow-up periods, 27% and 33% of participants quit smoking, respectively.

**Conclusion:** Smoking cessation rates were higher among participants with higher levels of education and lower levels of depressive symptoms. Smoking cessation interventions should be tailored to meet the needs of HIV-infected smokers and should be integrated into routine care to improve smoking cessation rates.
nicotine products. We identified smoking-related comorbidities based on laboratory results, clinical diagnoses, and treatments at baseline. We assessed associations between sociodemographic and clinical factors and prescription of smoking cessation medications using multivariable Cox proportional hazards analyses.

**Results:** Among 1,019 eligible PWH, 77% were men, 31% White, 48% Black/African American, 18% Hispanic/Latino, 46% aged 40 years and older. At baseline, 36% had smoking-related comorbidities including hypertension, chronic obstructive pulmonary disease (COPD)/emphysema, cardiovascular diseases (CVD), and cancers. During a median follow-up time of 2.6 years (interquartile range: 1.0–6.0), 342 (34%) were prescribed smoking cessation medications (Varenicline 106, Bupropion 53, nicotine products 273), and 241 (24%) quit smoking. In the multivariable analysis, having public insurance (hazard ratio [HR]: 1.90, 95% confidence interval [CI]: 1.34–2.69), care at a public clinic (HR: 1.57, CI: 1.15–2.13), anxiety (HR: 1.54, CI: 1.14–2.07), smoking-related cancer (HR: 2.69, CI: 1.00–7.26), and COPD/emphysema (HR: 1.69, CI: 1.05–2.71) were positively associated with prescription of smoking cessation medications (Figure).

**Conclusion:** Low percentages of smoking cessation medications prescription and smoking cessation among HOPS patients demonstrate gaps in primary prevention of smoking-related chronic diseases in PWH. Given the high burden of baseline comorbidities, enhanced smoking cessation efforts are warranted for protecting the health of this aging cohort of PWH.

Figure: Final Multivariable Cox Proportional Hazards Analysis Results of Factors Associated with Prescription of Smoking Cessation Medications, the HIV Outpatient Study, 2007-2021, N=1,019.

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**703 MITOCHONDRIAL HAPLOGROUPS AND WEIGHT GAIN AFTER INITIATING ART IN PATIENTS WITH HIV**

Juan Berenguer1, Inmaculada Jarrín2, José M. Bellón3, Cristina Díez2, María Á. Jiménez-Sousa4, Juan Carlos López5, José R. Blanco6, Joaquín Portilla7, Alvaro Mena8, María Novella9, David Dalmau10, Francesc Villaroya11, Pere Domingo12, Salvador Resino13,*

Cohort of the Spanish HIV Research Network (CoRIS) University Hospital Gregorio Marañón, Madrid, Spain, 1Centro Nacional de Epidemiología, Madrid, Spain, 2Hospital General Universitario Gregorio Marañón, Madrid, Spain, 3Centro Nacional de Microbiología, Madrid, Spain, 4Hospital San Pedro, Logroño, Spain, 5Hospital General Universitario de Alicante, Alicante, Spain, 6Complejo Hospitalario Universitario de La Coruña, La Coruña, Spain, 7Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain, 8Hospital Universitario Mutua de Terrassa, Terrassa, Spain, 9Universitat de Barcelona, Barcelona, Spain, 10Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, 11Instituto de Salud Carlos III, Madrid, Spain, 12*Presented at CROI by a nonauthor colleague

**Background:** Mitochondrial DNA (mtDNA) haplogroups have been associated with obesity among various populations and with weight gain following the switch to integrase strand transfer inhibitor (INSTI)-based antiretroviral therapy (ART) among people with HIV (PWH). We studied the association of mtDNA haplogroups with weight gain after ART in PWH.

**Methods:** Participants were ART-naïve PWH, recruited in the Spanish HIV Research Cohort, who started ART from 2014 onwards and had blood/DNA deposited in the cohort Biobank. The primary outcome was change in weight at 96 weeks after switching from ART. mtDNA genotyping was performed using the iPLEX Gold technology and Agena Bioscience’s MassARRAY platform (San Diego, CA, USA). Changes over time in weight and BMI were studied using adjusted linear mixed models (LMM).

**Results:** A total of 1,019 PWH were included. The mean weight gain over 96 weeks was 2.90 (95% CI: 2.54 – 3.26) kg. Factors associated with weight gain were female sex, birth in Sub-Saharan Africa, prior AIDS, CD4+ <200 cells/µl, HIV-RNA >10,000 copies/ml, negative HCV serology, and tenofovir alafenamide. The distribution of major mtDNA haplogroups was 376 (36.9%) HV, 158 (15.5%) UK, 138 (13.5%) JT, 45 (4.4%) INX, 187 (18.3%) non-N, and 115 (11.3%) N-undefined. The results of LMM adjusted by age, sex, BMI at baseline, country of birth, prior AIDS-defining conditions, CD4+ cell count, HIV-RNA viral load, type of ART regimen according to anchor drug, and NNRTI backbone showed an association between mtDNA haplogroup UK and a lower increase in weight and BMI at 96 weeks (Table).

**Conclusion:** The presence of the UK mtDNA haplogroup was associated with a lower increase in weight and BMI after ART in PWH. Our findings suggest that mitochondrial genomics plays a role in weight gain in this clinical context. Estimated means (95% CI) of weight (kg) and BMI at baseline and 96 weeks and increase according to major mtDNA haplogroups

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**704 WEIGHT CHANGE AFTER 48 WEEKS ON DOLUTEGRAVIR: A PROSPECTIVE STUDY OF PWH IN UGANDA**

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**Background:** Significant weight gain has been reported among people with HIV (PWH) initiating integrase inhibitor-based antiretroviral therapy (ART). We aimed to describe weight change over 48 weeks among PWH in Uganda who were transitioned from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART to a regimen containing tenofovir, lamivudine, and dolutegravir (TLD).

**Methods:** The DISCO cohort study followed PWH from a public-sector clinic in Mbarara, Uganda. Eligible participants were >18 years of age, on NNRTI-based, first-line ART for >6 months, and switched to TLD by clinic staff. Weight (in kilograms [kg]) and height (in meters) were measured at the enrollment, 24-week, and 48-week study visits. We describe weight change from baseline (week 0) to 48 weeks overall and stratified by sex, age, and regimen prior to switch. We then fit a multivariable linear regression model to examine correlates of weight increase, including regimen prior to switch, age, sex, education, and baseline body mass index.

**Results:** We analyzed data from 428 participants, with a mean age of 46.4 years and 43% women. Regimens prior to switch were 3TC/ATT/NVP (40%), 3TC/TDF/EFV (42%) or Other (18%). 95% of participants were virally suppressed at < 50 copies/mL at the time of switch to TLD. The mean body mass index prior to switch to TLD for men was 21.3 kg/m² (SD=2.9) and 25.2 kg/m² (SD=5.4) for women. We found no change in weight among men (0.08 kg, 95% CI: -0.45 – 0.62) and a modest change in weight among women (1.23 kg, 95% CI: 0.50-1.96). Mean weight at 0, 24 and 48 weeks by sex is shown in Figure 1. After adjustment for regimen, education, and age, being female remained significantly associated with an increase in body weight over 48 weeks.

**Conclusion:** Women experienced modest weight gain over 48 weeks after switching to TLD in Uganda. We found no weight change in men and no relationship between prior regimen and weight change. These findings suggest a heterogeneous impact of TLD on body weight across contexts.
WEIGHT GAIN FOLLOWING SWITCH TO DOLUTEGRAVIR AMONG ADULT HIV COHORTS IN WEST AFRICA

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IeDEA West Africa Collaboration

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Background: Following WHO recommendations, most countries have transitioned to Dolutegravir (DTG)-based regimens as first-line antiretroviral therapy (ART). Despite documented adverse metabolic effects from developed countries, limited information is available from resource-limited settings. We explored changes in body weight before and after switch to a DTG-based regimen and assessed the association between switch to DTG and significant weight gain (>10% increase) over a 12-month period in adults living with HIV (ALHIV) on ART in West Africa.

Methods: We first included all ALHIV followed in the IeDEA West Africa cohorts with a documented switch to DTG-based ART during 2019-2021. Participants had to be in care ≥36 months at the day of switch, with ≥1 weight measurement during the 24-months pre-switch period (P1) and the 12-months post switch period (P2). Weight change was estimated with a linear mixed model within P1 & P2, stratified by body mass index (BMI) class. A secondary analysis compared significant weight gain (>10%) in ALHIV on ART, prior and after the date of DTG introduction (site level dependent) between ALHIV who switched to DTG and those who did not at the date of database closure (control group), through a multivariate logistic regression with random effect analysis.

Results: In the first analyses, 5,294 ALHIV were included from three countries (Burkina, Côte d’Ivoire, Nigeria); 63% were women. Median age at day of switch was 48 years (IQR: 42-54) and median follow-up was 9 years (IQR: 6-12). Patients switched mainly from NNRTIs (83%) and PI-based (15%) ART regimens. Weight gain increased significantly during P2 compared to P1 period (P2). Weight change was estimated with a linear mixed model within P1 & P2, stratified by body mass index (BMI) class. A secondary analysis compared significant weight gain (>10%) in ALHIV on ART, prior and after the date of DTG introduction (site level dependent) between ALHIV who switched to DTG and those who did not at the date of database closure (control group), through a multivariate logistic regression with random effect analysis.

Conclusion: Most countries have transitioned to DTG-based ART regimens as first-line antiretroviral therapy (ART). Despite documented adverse metabolic effects from developed countries, limited information is available from resource-limited settings. We explored changes in body weight before and after switch to a DTG-based regimen and assessed the association between switch to DTG and significant weight gain (>10% increase) over a 12-month period in adults living with HIV (ALHIV) on ART in West Africa.

CHANGES IN BODY MASS INDEX WITH INTEGRASE INHIBITOR USE IN REPRIEVE

Emma Kileel1, Carlos Malvestutto2, Janet Lo3, Kathleen V. Fitch1, Carl J. Fichtenbaum1, Judith A. Aberg1, Markella V. Zanni3, Esteban Martinez6, Nwora Lance Okejo1, Princy Kumar1, Easu Joao1, Sara McCallum1, Pam S. Douglas4, Heather J. Ribaudo1, Steven K. Grinspoon5

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Background: Multiple studies have reported higher weight gain among people with HIV (PWH) with integrase strand transfer inhibitors (INSTIs), most notably in ART-naïve PWH and among women and Blacks/African Americans. However, knowledge gaps remain regarding long-term patterns of weight gain beyond the first two years after starting INSTI-based regimens in ART-experienced PWH.

Methods: An analysis of the effect of INSTIs on body mass index (BMI) was conducted in a large international cohort of PWH enrolled in REPRIEVE using mixed effect models with time-on-study as a continuous covariate. A stratified analysis was done evaluating 2-year change in BMI among participants on their entry ART regimen for 0-2 years, 2-5 years, and 5 or more years.

Results: Among a global cohort of PWH in REPRIEVE, the average rate of change in BMI per year was 0.19 (95% CI: 0.16-0.21) vs 0.14 (95% CI: 0.12-0.17) for participants on their entry regimen for more than 5 years. Among those on their entry regimen for 5 or more years, the average rate of change in BMI per year was 0.19 (95% CI: 0.16-0.21) vs 0.14 (95% CI: 0.12-0.17) for participants on their entry regimen for 5 or more years. Among those on their entry regimen for 2 years or less, the average rate of change in BMI per year was 0.14 (95% CI: 0.12-0.16) vs 0.12 (95% CI: 0.10-0.15) for participants on their entry regimen for 2 years or less. Among those on their entry regimen for 2-5 years, the average rate of change in BMI per year was 0.14 (95% CI: 0.12-0.16) vs 0.12 (95% CI: 0.10-0.15) for participants on their entry regimen for 2-5 years. Among those on their entry regimen for 2 years or less, the average rate of change in BMI per year was 0.14 (95% CI: 0.12-0.16) vs 0.12 (95% CI: 0.10-0.15) for participants on their entry regimen for 2 years or less. Among those on their entry regimen for 2-5 years, the average rate of change in BMI per year was 0.14 (95% CI: 0.12-0.16) vs 0.12 (95% CI: 0.10-0.15) for participants on their entry regimen for 2-5 years.

Conclusion: Most countries have transitioned to DTG-based ART regimens as first-line antiretroviral therapy (ART). Despite documented adverse metabolic effects from developed countries, limited information is available from resource-limited settings. We explored changes in body weight before and after switch to a DTG-based regimen and assessed the association between switch to DTG and significant weight gain (>10% increase) over a 12-month period in adults living with HIV (ALHIV) on ART in West Africa.

Changes in body mass index with integrase inhibitor use in REPRIEVE.

**Figure 1. Mean body weight for women and men with HIV at 0, 24 and 48 weeks after switching to TLD in Uganda**

**Figure 2. Mean body weight for women and men with HIV at 0, 24 and 48 weeks after switching to TLD in Uganda**

**Figure 3. Linear mixed model predictions of weight evolution from month-24 pre-switch to month-12 post switch to a DTG-based ART regimen in ALHIV by body mass index (BMI) class. The IeDEA West Africa collaboration.
DISCOVERING SUBGROUPS WITH LARGER WEIGHT GAIN WHEN TAKING DOLUTEGRAVIR

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Background: Clinical trials have shown that dolutegravir (DTG)-containing antiretroviral therapy results in larger weight gains than other antiretroviral therapies (ARTs). Not all people are expected to have the same risk for increased weight gain with DTG-containing ARTs. Discovering subgroups for whom DTG is more likely to cause substantial weight gain allows treatment recommendations to be tailored to a person’s risk profile.

Methods: We developed the Subgroup Discovery for Longitudinal Data (SDL-D) algorithm, a data-driven tree-based algorithm that combines modern machine learning and causal inference methods for discovering subgroups with heterogeneous treatment effects using longitudinal data. We applied the algorithm to electronic health records (EHRs) from the Academic Model Providing Access to Healthcare (AMPATH) partnership in Eldoret, Kenya to discover subgroups who are at higher risk of weight gain when on DTG-containing ARTs.

Results: A total of 84,445 individuals who were at least 18 years old, were initiating or on ART, and had data on or after July 1, 2016 with 1,178,016 unique clinic visits were included. The average causal effect on 1,000-day weight gain was 1.41 kilograms (kg) higher comparing always to never on DTG-containing ARTs (95% confidence interval (CI) [0.99, 1.82]). After searching over a large space of subgroups, the SDL-D algorithm identified gender as the primary source of heterogeneity in DTG effects and the average weight gain in 1,000 days when initiating or on ART, and had data on or after July 1, 2016 with 1,178,016 unique clinic visits were included. The average causal effect on 1,000-day weight gain was 1.41 kilograms (kg) higher comparing always to never on DTG-containing ARTs (95% confidence interval (CI) [0.99, 1.82]). After searching over a large space of subgroups, the SDL-D algorithm identified gender as the primary source of heterogeneity in DTG effects and the average weight gain in 1,000 days comparing always to never on DTG-containing ARTs was larger for females (2.25 kg, 95% CI [1.37, 3.12]) than males (0.87 kg, 95% CI [-0.04, 1.68]). The algorithm further split the female subgroup into whether they were older than or equal to 42.8 years old or not with older females having higher estimated weight gain. The figure shows the estimated weight trajectories among the subgroups.

Conclusion: We developed a novel algorithm that combines modern machine learning and causal inference methods to discover subgroups with heterogeneous treatment effects. We applied the algorithm to a large EHR database on people living with HIV in western Kenya. Our findings are consistent with prior research showing larger weight gains associated with DTG-containing ARTs. Gender was found to be the primary source of heterogeneity when we applied the first data-driven discovery of subgroups with larger weight gain when on DTG-containing ARTs.
709 VISCERAL FAT REDUCTION WITH TESAMORELIN ASSOCIATED WITH METABOLIC SYNDROME REVERSAL
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Medical Affairs Research Group
1University of Texas Southwestern, Dallas, TX, USA, 2Therapeutics, Inc, Dallas, TX, USA, 3Therapeutics, Inc, Chicago, IL, USA, 4Therapeutics, Inc, Seattle, WA, USA

Background: Among recent concerns surrounding weight gain in people with HIV (PWH) is the increasing incidence of Metabolic Syndrome (MetS). MetS, a component of which is central adiposity, is associated with increased risk of cardiovascular disease, stroke, and type 2 diabetes in PWH. Tesamorelin, a growth hormone releasing hormone analogue, was previously shown to reduce visceral adipose tissue (VAT) by over 15% in 26 weeks in PWH with lipohypertrophy. Due to the association between excess VAT and MetS, we investigated whether treatment with tesamorelin was also associated with changes in MetS classification.

Methods: We leveraged data from 2 Phase III trials of tesamorelin among PWH with excess VAT. Participants were randomized to receive tesamorelin (2 mg) or placebo subcutaneously daily for 26 weeks. In a per-protocol analysis of 400 participants assigned to receive tesamorelin, responders (R) were defined a priori by ≥8% reduction in VAT. Participants were evaluated and classified for MetS longitudinally by its 5 components - elevated waist circumference, high triglycerides, low HDL cholesterol, increased blood pressure, and elevated fasting blood glucose - according to both NCEP and IDF guidelines. Post hoc analyses were then performed to assess changes in MetS classification between responders and non-responders.

Results: The prevalence of MetS was high at baseline among study participants (37%) and did not significantly differ between responders (R) and non-responders (NR: R: 34.2%; NR: 43.8% by NCEP; p=0.077). However, following 26 weeks of tesamorelin treatment, the prevalence of MetS decreased in responders resulting in a significantly lower prevalence of MetS compared to non-responders (R: 30.8%; NR: 48.5%; p<.001). Differences in MetS status were driven predominantly by resolution of triglycerides (R: 26.3%; NR: 5.0%; p=0.005) and waist circumference (R: 25.6%; NR: 10.0%; p=0.031).

Conclusion: These data suggest that VAT reduction with tesamorelin is associated with a reversal of MetS classification among PWH. This is consistent with previous data indicating that visceral fat reduction is associated with improvements in metabolic parameters of PWH, and thus supports the use of tesamorelin in PWH with central adiposity to improve metabolic profiles.

711 CYTOKINE PROFILE IN DIFFERENT POST-COVID-19 CONDITION PHENOTYPES
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1IrsiCaixa Institute for AIDS Research, Badalona, Spain, 2Hospital Universitari Mutua de Terrassa, Barcelona, Spain, 3Infectious Diseases Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain, 4Fundació Lluita contra les Infeccions, Badalona, Spain

Background: Systemic hyperinflammation is key to the pathogenesis of severe, acute COVID-19. However, few studies have analysed inflammatory profiles in adults with mild/moderate COVID-19, or in those with post-acute sequelae of COVID-19 (PASC). We aimed to i) describe trajectories of cytokines in a prospective cohort of adults with mild to severe COVID-19, compared to uninfected, healthy controls and ii) identify early (< 4 weeks after illness onset) predictors of ongoing PASC and inflammation at 6 months after illness onset.

Methods: RECoVERED is a prospective cohort of adults with laboratory-confirmed SARS-CoV-2 infection between May 2020 and June 2021 in Amsterdam, the Netherlands. Serum was collected at weeks 4, 12 and 24. Participants completed monthly symptom questionnaires. PASC was defined as having at least one ongoing symptom that originated < 1 month of illness onset. Cytokine concentrations were analysed by human magnetic Lumex screening assay. We performed random forest regression to identify early predictors of PASC and raised CRP/IL-6 at 24 weeks, using Shapley additive explanation values as measures of importance for the different predictors.

Results: Of 349 RECoVERED participants, 186 (53%) had ≥2 serum samples and were included in current analyses. Of these, 101 (54%; 45/101 [45%] female, median age 55 years [IQR=45-64]) reported PASC at 12 weeks after illness onset, of whom none recovered by 24 weeks. We included 37 uninfected controls (17/37 [46%] female, median age 49 years [IQR=40-56]) at 4 weeks after illness onset; levels of IL10, IL1, IL17, IL1B, IL6 and TNFα were significantly elevated among participants infected with SARS-CoV-2 compared to controls. Ongoing PASC was independently associated with raised CRP at 24 weeks. Early raised IL10 and sCD14 levels and greater BMI at illness onset were the strongest predictors of PASC at 24 weeks. Those with higher early sCD14 or IL1 and TNFα levels were also more likely to have persistently raised CRP and IL6, respectively, at 24 weeks (Fig 1).

Conclusion: Differences in cytokine concentrations between individuals with COVID-19 and uninfected controls largely were greatest < 4 weeks after illness onset. In our study, ongoing PASC was associated with persistently elevated CRP at 24 weeks. Early immune dysregulation was, alongside BMI, an important determinant for persistent PASC. Further investigation of individuals with PASC and long-term aberrant cytokine levels may help improve our understanding of the condition.

Early (<4-0 week) predictors of PASC and CRP/IL-6 levels at 21-24 weeks after illness onset

710 INFLAMMATORY PROFILES ARE ASSOCIATED WITH LONG COVID 6 MONTHS AFTER ILLNESS ONSET
Elke Wynberg1, Alvin X. Han1, Lisa van Pul1, Irma Maurer2, Esther van Leeuwen3, Anouk Verveer4, Hugo van Willigen3, Menno D. de Jong5, Maria Prins1, Neeltje Kootstra1
1Public Health Department Amsterdam, Amsterdam, Netherlands, 2Amsterdam University Medical Center, Amsterdam, Netherlands, 3University of Amsterdam, Amsterdam, Netherlands

Background: At least 10% of SARS-CoV-2 infected patients suffer from persistent symptoms for >12 weeks, known as post-COVID-19 condition (PCC) or Long Covid. Reported symptomatology is diverse with >200 physical and neurological debilitating symptoms. Here, we analyzed pro-inflammatory cytokine levels as a potential mechanism underlying persistent symptomatology.

Methods: Clinical data and samples used belong to the KING cohort extension, which includes clinically well characterized PCC (N=358, 59 persistent symptoms evaluated), COVID-19 recovered and uninfected subjects. We used Gower distances to calculate symptom’s similarity between PCC and Ward’s hierarchical clustering method to identify different symptom patterns among PCC patients. Cytokine levels of randomly selected PCC, recovered and...
uninfected subjects (N=193) were measured on plasma samples collected >6 months after acute infection using the 30-Plex Panel for Lumineux. Mann–Whitney test was used to compare PCC vs recovered groups and Kruskal–Wallis t-test for >2 groups comparisons (PCC vs recovered vs Uninfected and within PCC clusters). FDR correction was applied for statistical significance (p-adj).

Results: Hierarchical clustering identified different PCC clusters according to their symptomatology, where PCC3 and PCC5 clusters showed higher prevalence of women (>80%) and more persistent symptoms, while acute COVID-19 was mild in >80% of the patients. We selected 91 PCC (belonging to each cluster), 57 recovered and 45 uninfected subjects for cytokine profiling (Table 1). 13 soluble markers were significantly elevated (IL-1β, Eotaxin, MIP-18, MCP-1, IL-15, IL-5, HGF, IFN-α, IL-1RA, IL-7, MG, IL-4 and IL-8) in PCC and recovered groups compared to uninfected subjects (all p-adj<0.04). In addition, PCC subjects tended towards higher levels of IL-1RA compared to recovered group (p-adj=0.07). Within PCC clusters, FGF-basic and RANTES were elevated while IL-2 and IL-4 were decreased in PCC3 and PCCs compared to the other PCC clusters (all p-adj<0.04). TNF-α, IP-10, G-CSF and MIP-1α were decreased in PCC3 and PCC5 not reaching statistical significance (all p-adj=0.07).

Conclusion: Some cytokines remained altered in all SARS-CoV-2 infected subjects independently of persistent symptoms after 6 months from acute infection. Differences between PCC and recovered individuals are limited after correction. Importantly, PCC cytokine profiles showed differences between clusters, which suggests different PCC subgroups with distinct etiologies.

Subjects Characteristics

<table>
<thead>
<tr>
<th>No of PCC clusters</th>
<th>PCC1</th>
<th>PCC2</th>
<th>PCC3</th>
<th>PCC4</th>
<th>PCC5</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>20</td>
<td>36</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>193</td>
</tr>
<tr>
<td>Gender</td>
<td>69.5%</td>
<td>61.1%</td>
<td>70%</td>
<td>73.7%</td>
<td>65%</td>
<td>56.9%</td>
</tr>
<tr>
<td>Age (mean, SD)</td>
<td>49.2 (17.9)</td>
<td>49.5 (17.8)</td>
<td>57.7 (17.9)</td>
<td>57.2 (17.9)</td>
<td>57.7 (17.9)</td>
<td></td>
</tr>
</tbody>
</table>

Infectious SARS-CoV-2 may trigger autoimmune disease through T-cell-mediated autoimmune response through molecular mimicry-cross-reactive T-cell recognition or bystander T-cell activation. Autoantibodies have been detected in patients with COVID-19 and some human proteins have homologous regions with SARS-CoV-2 peptides that could function as autoantigens. While there are scattered reports of various autoimmune diseases diagnosed after COVID-19, the risk is not known.

Methods: TrillnetX (a global federated health research network providing access to electronic medical records across 72 large healthcare organizations) was utilized to define a cohort of adults 18 years or older seen on or after January 1, 2020 with at least one follow-up visit after an index date. Exposure was defined as COVID-19 diagnosis by ICD10 code or positive laboratory test. Controls did not have COVID-19 (by the same criteria) and were propensity score-matched to patients who had COVID-19 by age and female sex. Index date was the date of COVID-19 diagnosis or first provider visit for any reason during the study period for controls. Outcomes (see table) were assessed starting one month after index date (to exclude prior undiagnosed autoimmune disease) until one year after. Patients with a specific outcome prior to the index date or within one month after the index date were excluded from the analysis for that outcome. Incidence by COVID-19 exposure status and risk ratios for each outcome were assessed.

Results: 4,016,472 patients were included (2,008,236 in both groups). Overall, mean (SD) age was 49.2 (17.9) and 57.7% were female. Patients who had COVID-19 were more likely to be white (63 vs 56.9%; p < 0.001). Rheumatoid arthritis, psoriasis and type 1 diabetes mellitus had the highest incidence after COVID-19 (0.24, 0.22 and 0.19%, respectively). While the incidence of most of the autoimmune diseases assessed were low in both groups, the risk ratios for all but one condition (Grave’s) showed statistically significant higher risk in patients after COVID-19 than in those without COVID-19 (see table). Risk ratios were highest for polyarteritis nodosa (4.43, 3.27-6.01), reactive arthritis (3.56, 2.05-6.2) and ANCA-associated vasculitis (3.36, 2.6-4.3).

Conclusion: Autoimmune diseases were more likely to be diagnosed within the first year after COVID-19 than in age-, sex-matched controls. Future work will assess the validity of autoantibodies in predicting autoimmune disease after COVID-19.

Risk of Autoimmune Disease Within One Year of COVID-19 Diagnosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>1.2 (1.26-1.38)</td>
</tr>
<tr>
<td>Axial or Peripheral Spondyloarthritides</td>
<td>1.3 (1.1-1.55)</td>
</tr>
<tr>
<td>Reactive Arthritis</td>
<td>3.56 (2.06-6.3)</td>
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<tr>
<td>Adult Onset Still Disease</td>
<td>2.8 (0.55-5.05)</td>
</tr>
<tr>
<td>Giant Cell Arthritis</td>
<td>1.3 (1.04-1.60)</td>
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<tr>
<td>Polymyositis</td>
<td>4.4 (1.27-6.01)</td>
</tr>
<tr>
<td>Raynaud's Syndrome</td>
<td>2.20 (1.39-3.65)</td>
</tr>
<tr>
<td>ANCA Associated Vasculitis</td>
<td>3.30 (2.70-4.34)</td>
</tr>
<tr>
<td>Cutaneous Vasculitis</td>
<td>2.12 (0.87-5.39)</td>
</tr>
<tr>
<td>Anti-GM1 Disease</td>
<td>2.75 (1.70-6.78)</td>
</tr>
<tr>
<td>Sytemic Lupus-Erythematosus</td>
<td>3.15 (1.61-6.14)</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1.24 (1.02-1.57)</td>
</tr>
<tr>
<td>Systemic Sclerosis</td>
<td>3.54 (3.2-3.83)</td>
</tr>
<tr>
<td>Postinfection</td>
<td>5.41 (3.1-4.27)</td>
</tr>
<tr>
<td>Sjogren Syndrome</td>
<td>2.27 (1.61-3.17)</td>
</tr>
<tr>
<td>Crohn's Disease</td>
<td>1.52 (1.47-1.65)</td>
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<tr>
<td>Ulcerative Colitis</td>
<td>5.17 (4.17-6.28)</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>1.34 (1.09-1.63)</td>
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<tr>
<td>Mixed Connective Tissue Disease</td>
<td>2.43 (2.3-2.58)</td>
</tr>
<tr>
<td>Diabetes Mellitus Type 1</td>
<td>1.65 (1.6-1.78)</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>1.58 (1.5-1.63)</td>
</tr>
<tr>
<td>Autoimmune Hepatitis</td>
<td>1.19 (1.0-1.39)</td>
</tr>
<tr>
<td>Autoimmune Thymididosis</td>
<td>1.18 (1.12-1.25)</td>
</tr>
<tr>
<td>Grave's Disease</td>
<td>0.973 (0.89-1.15)</td>
</tr>
</tbody>
</table>

Analysis was performed on the cohort after propensity score matching by age and sex. Patients with an outcome prior to the study window were excluded from the analysis of that outcome.

INCIDENT NEW ONSET DIABETES AFTER COVID-19 INFECTION: A NATIONWIDE MULTICENTER COHORT

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Background: It is known that survivors of acute SARS-CoV-2 infection can experience a complex disease known as post-acute sequelae of COVID-19 (PASC). The clinical manifestations of acute COVID-19 have been well characterized however less is known about the risk of new onset diabetes mellitus (DM) in the post-acute phase of COVID-19.

Methods: An adult cohort with confirmed COVID-19 (by diagnosis or positive test) and without COVID-19 was sampled from a large national health research network between January 1st, 2020 and July 8th, 2022. We investigated the outcomes of a new diagnosis of DM (type i or ii) occurring after COVID-19 through 12 months after infection. Risk estimates [incidence, relative risk (RR), attributable risk] were used to describe the probability of incident post-COVID diabetes. Hazard ratios and 95% confidence intervals were used to describe risk factors associated with new diabetes.

Results: The 3-month probability of new diabetes was 2.48/1,000 among COVID+ and the relative risk (RR) of new diabetes was highest at 12 months [8.94 (8.54, 9.36)]. Vitamin D deficiency [HR: 1.52 (95% CI: 1.42, 1.63)] was associated with increased risk of new diabetes starting at 3 months after acute infection using the 30-Plex Panel for Lumineux. Mann–Whitney test was used to compare PCC vs recovered groups and Kruskal–Wallis t-test for >2 groups comparisons (PCC vs recovered vs Uninfected and within PCC clusters). FDR correction was applied for statistical significance (p-adj).

Conclusion: Autoimmune diseases were more likely to be diagnosed within the first year after COVID-19 than in age-, sex-matched controls. Future work will assess the validity of autoantibodies in predicting autoimmune disease after COVID-19.
WORSE ARTERIAL STIFFNESS, AND NOT ENDOTHELIAL DYSFUNCTION, IS ASSOCIATED WITH PASC

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Background: COVID-19 survivors can experience lingering symptoms known as PASC that appear in different phenotypes. The etiology remains elusive and endothelial dysfunction has been postulated as a main driver of PASC.

Methods: Prospective cohort including COVID+ and COVID- with (COVID+PASC+) or without (COVID+PASC-) PASC. We measured endothelial function using Endopat, an FDA approved test, with derived reactive hyperemic index RHI (endothelial dysfunction ≤1.67) and arterial elasticity (augmentation index at 75 bpm or AI@75; lower = better). PASC symptoms were categorized into three non-exclusive phenotypes: Cardiopulmonary CP (post-index standardized at 75 bpm or AI@75; (lower = better). PASC symptoms were initially impaired with the proviso of attrition although random. Olfactory performance declined over time (p<.0001). IFN-γ, IL-1Rα, IL-13 and TNF-α increased across time, p<.03-p<.0005. TNF-α shown no relationship with the kynurenine pathway, but an association of 12p70, 13, and MCP-1, TNF-α and INF-γ), analyzed as Log transformed and immune markers (Interleukin-IL panel: 1-β, 1Rα, 4, 5, 6, 8, 10, 12p40, 24-month. We tested peripheral neurobiomarkers (NFL, GFAP, S100B, GM-CSF) and IFN-γ showed a time covariance with poorer olfaction performance. Olfactory performance declined over time (p<.0001), which was dependent on interaction).

Results: At 2 months post-diagnosis 30% had impaired olfaction and those who had acute severe disease were more likely to be impaired (54% versus 47%; p=.009). 21%, 31% and 37% had impaired olfaction at 4, 12 and 24-months. Olfactory performance declined over time (p<.0001), which was dependent on interaction).

Conclusion: Post-acute mild to moderate acute COVID-19 across 2 years

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Background: Previous longitudinal studies (n=6) of objective olfaction performance post-acute COVID-19 have a maximum follow-up of 6-month and do not often test biomarkers. Although olfactory dysfunction appears to improve within two months of symptom onset, 4/6 longitudinal studies show persistent olfactory impairment.

Methods: PCR-confirmed COVID-19 patients in the prospective ADAPT cohort (Sydney, Australia) were assessed across 18 acute symptoms and hospitalization status: 40% mild, 50% moderate, 10% severe/hospitalised – none deceased. Blood samples were taken 2 (N=179), 4 (N=148) and 12-month (N=118) post-diagnosis. Blood samples were taken 2 (N=179), 4 (N=148) and 12-month (N=118) post-diagnosis. The NIH Olfactoid Identification Test (OIT) and the Cogstate brief cognitive battery were performed. 58 also had an olfaction test at 24-month. The OIT raw data were transformed into demographically-corrected T-scores. Olfactoid attrition was completely random and only initial age (40±15 versus 47±15) differed between patients lost to follow-up and those in the study at 24-month. We tested peripheral neurobiomarkers (NFL, GFAP, S100B, GM-CSF) and immune markers (Interleukin-IL panel: 1-β, 1Rα, 4, 5, 6, 8, 10, 12p40, 12p70, 13, and MCP-1, TNF-α and INF-γ), analyzed as Log transformed and elevated/normal range using published references. Our previous analyses had shown no relationship with the kynurenine pathway, but an association of impaired olfaction and impaired cognition at 2-month only. Linear mixed effect regressions with time effect (months) tested olfaction trajectories (random subject effect) and their association with the biomarkers (main and time interaction).

Results: At 2 months post-diagnosis 30% had impaired olfaction and those who had acute severe disease were more likely to be impaired (54% versus 26%, p<.009). 21%, 31% and 37% had impaired olfaction at 4, 12 and 24-months. Olfactory performance declined over time (p<.0001), which was dependent on interaction (Fig 1). Neurobiomarkers were within the normal range. IFN-γ increased across time, p<.03-p<.0005. TNF-α and INF-γ showed a time covariance with poorer olfaction performance. Olfactory performance decline may be mediated by upregulated immune parameters which are distinct from those driving cognitive changes.
OLFACTORY FUNCTION AND BRAIN STRUCTURAL EFFECTS FOLLOWING SARS-CoV-2 INFECTION

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*Presented at CROI by a nonauthor colleague

Background: SARS-CoV-2 infection is accompanied by acute olfactory disturbance in as high as 70% of cases. This loss is associated with decreased olfactory bulb volume. As time passes, the anosmia tends to subside, but the OB volume decrease does not. Volume reductions in primary and secondary olfactory cortex are also seen following SARS-CoV-2 infection. Nevertheless, concurrent SARS-CoV-2 infection effects on olfactory discrimination, olfactory bulb volume, primary olfactory cortex and its targets have not been investigated. To explore this possibility, we measured olfactory discrimination, olfactory bulb volume, primary olfactory cortex and basal ganglia volume in patients who had SARS-CoV-2 infection more than 12 weeks previously, who were then divided into COVID and long-COVID groups on the basis of self-reported fatigue and concentration complaints.

Methods: This cross-sectional study included 25 post-infection and 19 demographically-matched, no-COVID control participants, we investigated the effects on olfaction using NIH Toolbox Odor Identification Test and the Monell Smell Questionnaire. GM structure was assessed with voxel-based morphometry and manual delineation of high resolution (1mm³), T1- and T2-weighted MRI data. Linear regression was used to model group effects on GM structure, adjusting for age, sex, education and total intracranial volume. CAT12/SPM12 and R were used for image processing and statistical modeling.

Results: The NIH Toolbox Odor Identification Test failed to show differences among the groups. In contrast, the Monell Smell Questionnaire revealed persistently diminished and distorted smell in 50% of the long-COVID sample. Olfactory bulb volume was lower in the long-COVID group (p<0.02). Primary olfactory cortex volume was reduced in the long-COVID group (p=0.004). Caudate volume was also lower in the long-COVID group (p=0.04).

Conclusion: Conclusions. In the absence of olfactory discrimination problems, long-COVID, but not COVID, patients experience persistent olfactory loss and distortion. These perceptual problems are associated with lower olfactory bulb, primary olfactory cortex, and caudate volume, suggesting that the effects of SARS-CoV-2 infection can extend beyond the olfactory periphery in some cases to affect central targets.

SEX MODIFIES THE EFFECT OF COVID-19 AND PASC STATUS ON ARTERIAL STIFFNESS

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Background: Sex differences in immunological responses to COVID-19 infection and mechanisms that may contribute toward post-acute sequelae of SARS-CoV-2 (PASC) have been reported. However, evidence on the effects of COVID infection on vascular dysfunction and PASC are limited.

Methods: FDA approved EndoPAT device was used to measure endothelial function (Reactive Hyperemia Index (RHI)) and arterial stiffness (Augmentation Index standardized at 75 beats/min (AI@75; higher AI = worse arterial elasticity)) in an adult cohort (age ≥18 years) with a history of COVID-19 infection (COVID+ or confirmed SARS-CoV-2 antibody negative (COVID-)). Generalized linear regression was used to compute estimates of RHI and AI@75. Adjusted models included age, sex, race, blood pressure, lipids, body mass index (BMI), smoking status, and pre-existing comorbidities. Two-way interactions were used to determine if the effects of COVID or PASC status on endothelial function depends on age, sex, race, smoking status, or prevalent comorbidities.

Results: 61.99% (n=305) of study participants were COVID+ and 38.01% (n=193) were COVID-. Among COVID+, 57.22% (n=107) were female, 31.72% (n=59) were non-white race, and the average age was 46.64±13.79 years. COVID+ participants had a smaller proportion (38.03%) of female sex (p=0.001), lower BMI (COVID+ (30.79±8.59 kg/m²) vs. COVID- (27.76±5.89 kg/m²); p<0.001), and higher proportion of smokers (COVID+ (30.99%) vs. COVID- (27.76%); p<0.001). The average follow-up was 349.68±276.76 days and 109 (22.15%) COVID+ experienced PASC. 42.48% (n=80) of COVID+ and 41.64% (n=127) of COVID- had RHI<1.67 (p=0.8). The average AI@75 among COVID+ without PASC was 3.63±16.24, with PASC was 10.5±14.72, and 3.11±15.97 among COVID-. Among COVID-, male sex (10.75±15.3; <0.001) had the lowest AI@75 compared to COVID+ without PASC, male sex (p<0.001), 13.81±2.11 higher AI@75 compared to COVID+ with PASC, male sex (p=0.001), and 4.97±2.28 higher AI@75 compared to COVID+, female sex (p=0.03). Sex was not associated with RHI or modified the effect of COVID or PASC status on endothelial function.

Conclusion: The effect of COVID and PASC status on arterial stiffness depends on sex. Female sex is associated with increased arterial stiffness (worse arterial elasticity) in the post-acute phase of COVID-19.
INCIDENCE OF LONG COVID SYMPTOMS IN PATIENTS PREVIOUSLY HOSPITALIZED FOR COVID-19
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Background: Long COVID, also known as post-acute sequelae of COVID (PASC), affects more than 144 million people globally. While there is no broadly accepted consensus on a definition for the term “long COVID,” studies have found symptoms persist or begin weeks or months after the end of SARS-CoV-2 infection. This study assessed the incidence of codes found in medical claims and hospital chargemaster data that were consistent with long COVID symptoms commonly found in the literature.

Methods: Using the HealthVerity database, which provides closed claims and linked hospital chargemaster data on more than 25 million US patients, we examined patients aged 12 and above who had ≥ 1 visit to a PAC-19 clinic from May 1, 2020 to September 30, 2021 with a diagnosis of COVID-19 who had at least 365 days of closed medical claims enrollment prior to index hospitalization admission and 90 days after admission, and who did not have a long COVID diagnosis (ICD-10-CM U09.9) prior to the index hospitalization. Patients were allowed to have symptoms prior to hospitalization. The assessment period for the outcomes, which included 10 symptoms, was 90 days to 270 days after the date of hospitalization. Incidence rate per 100 person-years was calculated as the number of patients with the outcome divided by total person-time contributed (90 days after admission to the minimum of the following: outcome, inpatient death, disenrollment, end of data (April 30, 2022), or 270 days after admission).

Results: The dataset included 3,661,303 patients with an inpatient hospitalization during the study period. The final study cohort included 44,922 patients hospitalized with COVID-19, 20,627 of whom experienced at least one of the long COVID symptoms. Anosmia and dysgeusia were the rarest events captured in medical claims. More commonly found symptoms were joint pain, fatigue, and breathlessness (see Table). Conclusion: This study examined diagnosed symptoms commonly found post-hospitalization among COVID-19 patients and reported the incidence of these symptoms in a representative population. The start period of long COVID used in this study (90 days post hospitalization) is consistent with the WHO definition of long COVID. In the absence of an understanding of the pathophysiology of long COVID, the use of diagnosed symptoms to define long COVID has the advantage of ease of use and availability of data. Further studies of additional symptoms and predictors of long COVID are needed.

Number of diagnosed long COVID symptoms and rate per 100 person-years among patients who had been hospitalized for COVID-19

<table>
<thead>
<tr>
<th>Symptom</th>
<th>N</th>
<th>Rate per 100 person-years*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste disturbance/dysgeusia/anosmia</td>
<td>40</td>
<td>0.20</td>
</tr>
<tr>
<td>Smell disturbance/anosmia</td>
<td>45</td>
<td>0.22</td>
</tr>
<tr>
<td>Muscle pain/myalgia</td>
<td>1,235</td>
<td>6.64</td>
</tr>
<tr>
<td>Headache</td>
<td>1,373</td>
<td>6.89</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>1,983</td>
<td>10.04</td>
</tr>
<tr>
<td>Cough</td>
<td>3,492</td>
<td>18.33</td>
</tr>
<tr>
<td>Chest pain</td>
<td>6,805</td>
<td>35.86</td>
</tr>
<tr>
<td>Joint pain/arthralgia</td>
<td>7,679</td>
<td>42.54</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8,657</td>
<td>45.04</td>
</tr>
<tr>
<td>Dyspnea/breathlessness</td>
<td>8,356</td>
<td>46.93</td>
</tr>
<tr>
<td>Any of the above symptoms</td>
<td>20,827</td>
<td>150.92</td>
</tr>
</tbody>
</table>

*Premature is calculated as the number of days between day 90 post-admission and the minimum of the following: outcome, inpatient death, disenrollment, day 270 post-admission, or end of data (April 30, 2022).

15-MONTH ATTRIBUTES OF POST-COVID SYNDROME IN THERAPEUTICALLY VACCINATED OUTPATIENTS
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Background: In the third year of the coronavirus disease 2019 (COVID-19) pandemic, long-term post-COVID syndrome (PACS) following severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infections poses the significant challenge for patients and health systems globally. Whilst COVID-19 vaccinations prior to SARS-CoV-2 infection reduce the risk of PCS, the role of therapeutic vaccination in PACS recovery remains controversial. We present a 15 months longitudinal, prospective observational cohort study to examine long-term clinical courses, PCS recovery with and without vaccination as well as humoral immune responses in initially unvaccinated PCS patients.
**Methods:** A total of 227 COVID-19 convalescents of our initial mild COVID-19 outpatient cohort (N=958) from which longitudinal data was available were included in this study. PCS was defined according to the WHO definition. 76.7% (174/227) of individuals received at least one vaccination between 10 and 15 months after first SARS-CoV-2 infection. Receptor binding domain (RBD)-specific SARS-CoV-2 immunoglobulin G (IgG) and distinct symptom phenotypes (P) were longitudinally assessed for 15 months. Using binomial regression models, odds ratios (OR) with 95% confidence interval (95%CI) of descriptive, longitudinal variables associated with long-term PCS were calculated.

**Results:** 35.8% (82/227) and 31.3% (71/227) of patients had PCS at months 10 and 15 (figure 1A). SARS-CoV-2 IgG titer were equally distributed over time among age groups, sex, and PCS. PCS occurred in 30.5% (53/174) of vaccinated and 34.0% (18/53) of unvaccinated patients. Between 6 and 10 months (ΔT2/T3: not yet vaccinated) and 10 and 15 months (ΔT3/T4: vaccinated) after symptom onset (figure 1B), a comparable fraction of PCS patients recovered (ΔT2/T3: 19.5% ± 20.0% and ΔT3/T4: 20.0%). Fatigue/dyspnea (P2) and not anosmia/ageusia (P1) constituted PCS at month 15 (P2 23.2% versus P1 1.4%). Headache (P4) and diarrhea (P5) at baseline were risk factors for PCS at months 15, respectively (P4: OR 2.01 (95%CI 1.11-3.52), p= .018; P5: OR 3.01(95%CI 1.44-5.94), p = .002).

**Conclusion:** Our results indicate, that distinct symptom phenotypes can constitute and predict long-term PCS 15 months after mild COVID. Recovery of PCS was observed similarly in both therapeutically vaccinated and unvaccinated patients. Thus, development of targeted PCS therapeutics is needed to improve patient care and future epidemiological investigations.

**Figure 1**

**POST-COVID SYMPTOMS IN VACCINATED AND UNVACCINATED COVID-19 PATIENTS

**A** Absolute (A) and percentage (%) distribution of patients with post-COVID syndrome (PCS) at the respective visit (T1, T2, T3, T4). T1: day of delivery to PCS patients between T2 and T3 (ΔT2/T3) without vaccination and between T3 and T4 (ΔT3/T4) following vaccination. Reproduced, with permission, from Health organisation.

**B** Recovery of PCS patients at month 6 (T2) after symptom onset

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**SYMPTOMS AND BIOMARKERS OF LONG COVID IN PEOPLE LIVING WITH AND WITHOUT HIV**

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**Background:** HIV is a risk factor for severe acute COVID-19, but it is unknown whether HIV is a risk factor for long COVID.

**Methods:** We conducted a prospective observational cohort study of US adults with HIV (PWID) and HIV-seronegative adults with first SARS-CoV-2 infection within 4 weeks together with people who never had COVID-19. At enrolment, participants recalled the presence and severity of 49 long COVID-associated symptoms in the month prior to COVID-19. The same symptom survey was administered at 1, 2, 4, and 6 months post-COVID or post-enrolment for never-COVID participants. Post-COVID participants donated blood 1 and 4 months post-COVID, and never-COVID participants donated blood 0-1 times. Antibody
titers to 18 coronavirus antigens and levels of 30 cytokines and hormones were quantified (Messa Scale Discovery). The Mann Whitney U test was used to compare continuous variables between groups, and Pearson's chi-squared test for categorical variables. Spearman correlation analyses were used to build networks of associations between cytokines and symptoms.

**Results:** 341 participants enrolled between June 2021 and September 2022. Of these, 73 were PWH post-COVID, 121 were HIV-seronegative post-COVID, 78 were PWH never-COVID, and 69 were HIV-seronegative never-COVID. Over 85% of participants were vaccinated prior to COVID-19. Most participants with HIV were male sex at birth (83% post-COVID, 59% never-COVID), on ART (>95%), with median CD4 counts >500.

Over 60% of participants reported 1+ new or worsened symptoms 2-6 months post-COVID, with higher percentages in PWH at 2 months post-COVID (< p<0.05). PWH were more likely to report body ache, pain, confusion, memory problems, and thirst and had higher levels of creatine phosphokinase post-COVID than HIV-seronegative people. SARS-CoV-2 and non-SARS human coronavirus antibody titers did not differ between PWH and HIV-seronegative post-COVID participants.

Cytokine associations with each other (network density) were significantly enriched at 1 month post-COVID in both PWH and HIV-seronegative people, with significantly less enrichment at 4 months post-COVID and in never-COVID participants. Levels of four analytes (cortisol, C5a, TGF-β1, and TIM-3) associated with specific symptoms of long COVID.

**Conclusion:** PWH may experience more symptoms post-COVID with a slightly different symptom profile than people without HIV. Inflammatory networks were active in PWH and people without HIV at 1 month post-COVID.

### SEVERITY CLUSTERS AND LIKELIHOOD OF RECOVERY FROM POST-COVID-19 CONDITION

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**Background:** The Post-COVID-19 Condition (PCC) is a novel, long-lasting, poorly understood and highly disabling post-viral syndrome, which poses enormous healthcare, economic and socio-political challenges. Lack of validated biomarkers forces clinical management to be based on clinical definitions, which are imprecise. In the clinic, symptoms tend to present in clusters, which have yet to be properly defined. Also, it is unclear how often PCC resolves, and which factors influence PCC resolution.

**Methods:** To delineate PCC presentation clusters and explore factors related with PCC resolution, we performed a 2-year prospective cohort study in individuals who recovered from acute COVID-19 regardless of its acute and post-acute severity. All subjects were systematically followed in the outpatient post-COVID-19 clinic of a tertiary care hospital in Spain. PCC was defined as per the WHO 2021 definition. Persistent symptoms were those present >3 months after acute COVID-19, and lasting for >2 consecutive months. PCC recovery was the absence of PCC symptoms during >3 consecutive months. Symptom clusters were identified using Gower's distance matrices, dendograms, PCA and Silhouette techniques. Factors associated with PCC recovery were identified using a directed acyclic graph approach.

**Results:** 548 subjects were included; 341 (62%) had PCC. The latter were mostly females (69.8%) with mean age of 47.9 (SD 12.2) years. Only 38.1% required hospitalization and 9% required high-flow oxygen during acute COVID-19. Their most frequent comorbidities were allergy (31.4%), obesity (24.8%), dyslipidemia (24.0%) and hypertension (19.6%). At least 3 symptom clusters with additive symptoms were identified: considering only symptoms present in >25% of subjects, Cluster A was enriched in fatigue and dyspnea; Cluster B had Cluster A symptoms plus headache, arthralgia and neurocognitive complaints; Cluster C had Cluster B symptoms plus chest pain and tachycardia. PCC recovery was achieved by 26 (7.6%) individuals over 2 years. Male sex (RR 3.01; CI 1.4-6.3), ICU admission (RR 7.78; CI 2.6-23.2), metabolic comorbidity (RR 2.07; CI 1.1-4.1), and mild acute COVID-19 (RR 2.70; CI 1.1-4.6) increased the likelihood of PCC recovery. Conversely, subjects with muscle pain, impaired attention, dyspnea, and tachycardia were less likely to recover from PCC (RR 0.26; CI 0.13-0.52).

**Conclusion:** At least 3 severity clusters can be identified in the PCC. Over the first 2 years, only a minority of subjects fully recover from PCC.

### IMMUNE STATUS AND SARS-CoV-2 VIRAL DYNAMICS

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**Background:** Immunocompromised persons are disproportionately affected by severe SARS-CoV-2 infection, but immune compromise is heterogeneous, which may impact viral dynamics. We hypothesized that higher degrees of compromised immunity are associated with higher viral shedding and slower viral clearance in the absence of COVID-19 therapeutics.

**Methods:** Participants enrolled in ACTIV-2/A5401, a platform trial for COVID-19 therapeutics in non-hospitalized adults within 10 days of symptom onset, received either an active treatment or placebo between 8/2020 and 7/2021. Participants were categorized based on the extent of immunosuppression into...
none, mild, moderate and severe categories at enrollment (day 0). Longitudinal anterior nasal (AN) and plasma SARS-CoV-2 levels were measured with a quantitative PCR assay. Regression models assessed associations between immunocompromise severity and viral levels (VL) at day 0, and longitudinally among those on placebo with quantifiable RNA at day 0. Multivariate analyses adjusted for demographics and symptom duration and vaccination status at day 0.

Results: Immunocompromised (mild 383, moderate 159, severe 35) and immunocompetent (1956) participants had comparable symptom durations at day 0 (median 6 days) and most were unvaccinated (~95%). AN VL at day 0 was higher in the moderate/severe group compared to the immunocompetent group (adjusted difference in means: 0.47 log_{10} copies/mL, 95% CI 0.12, 0.83). While AN VL decayed at similar rates among all groups from day 0 to 3, there was a trend towards higher cumulative AN VLs across the 28-day follow-up in the moderate/severe group compared to immunocompetent group (adjusted fold difference in VL AUC 1.63, 95% CI 0.95, 2.77). The mild group showed no differences in day 0 VL or AUC compared to the immunocompetent group. The frequency of detectable plasma SARS-CoV-2 RNA was similar at day 0 across all groups (overall 21%), but there appeared to be a higher proportion of immunocompromised participants with detectable plasma viral RNA at day 7 (moderate/severe 2/23 (9%), mild 5/44 (11%)]) compared to the immunocompetent group (8/282, 3%).

Conclusion: Before emergence of Omicron and widespread vaccination, moderate/severe immunocompromised status was associated with higher nasal viral levels at study enrollment and showed a trend towards higher cumulative AN viral load, and all immunocompromised groups appeared to have more persistent plasma viremia during follow-up.

Prolonged Viable Viral Shedding in Immunocompromised Patients with COVID-19
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Background: Immunocompromised patients with COVID-19 tend to shed viable virus for a prolonged period. Therefore, for moderately or severely immunocompromised patients with COVID-19, CDC recommends an isolation period of at least 20 days and ending isolation in conjunction with serial testing and consultation with an infectious disease specialist. However, data on viral kinetics and risk factors for prolonged viral shedding in these patients are limited.

Methods: From February 1, 2022 to April 1, 2022, we collected weekly saliva samples from immunocompromised patients with COVID-19 admitted to a tertiary hospital in Seoul, South Korea. Genomic and subgenomic RNAs were measured, and virus culture was performed.

Results: A total of 41 patients were enrolled; 29 (70%) were receiving treatment for COVID-19, 12 (30%) had undergone solid organ transplantation. Of the 41 patients, 14 (34%) had received 3 doses or more of COVID-19 vaccines. Real-time RT-PCR revealed that 7 (17%) were infected with Omicron BA.1, and 33 (80%) with Omicron BA.2. The median duration of viable virus shedding was 4 weeks (IQR 3-6). Patients undergoing B-cell depleting therapy shed viable virus for longer than the comparator (p=0.01). Multivariable analysis showed that 3-dose or more vaccination (HR 0.33, 95% CI 0.12 – 0.95, p = 0.04) and B-cell depleting therapy (HR 12.50, 95% CI 2.44 – 100.00, p = 0.003) independently affected viable virus shedding of SARS-CoV-2.

Conclusion: Immunocompromised patients with COVID-19 shed viable virus for a prolonged period. Therefore, for moderately or severely immunocompromised patients with COVID-19, CDC recommends an isolation period of at least 20 days and ending isolation in conjunction with serial testing and consultation with an infectious disease specialist. However, data on viral kinetics and risk factors for prolonged viral shedding in these patients are limited.

Suicidal Risk Among People with HIV After Lockdown Due to COVID-19 Pandemic
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Background: People with HIV (PWH), are known to be at increased risk for mental disorders and suicidal risk (SR) when compared to general population. Despite the fact that suicide represents the 7th cause of death, routine assessment of SR among PWH is uncommon. The COVID19 pandemic caused a significant increase in mental disorders in the general population. Few studies have described the impact of the pandemic on SR in PWH. We aim to describe the trend in SR prevalence in PWH who attended our HIV clinic before (2018 and 2019), during (2020) and after (2021) the lock-down due to COVID19, as well as the factors associated.

Methods: PWH (adults) receiving care in a tertiary facility in Mexico City during 2018-2021 were included. Since 2018, a questionnaire based on the Columbia Suicide Severity Rating Scale, has been routinely applied to screen for SR in all HIV clinic visits. We described and compared the sociodemographic characteristics of those classified with SR vs those without SR. We estimated SR using the responses of patients assessed through the questionnaire, by calendar year. We evaluated the potential association of calendar year on the SR probability using a mixed effects logistic regression model, including sex, being undetectable, CD4count and efavirenz (EFV)-based ART use, at evaluation date; cumulative time in ART, year and route of HIV transmission as fixed effects.

Results: During the study period, 2275 patients were included; 93% (n=2130) were routinely evaluated for SR at least once. Fifty-nine (5%) had SR. Those with SR, compared with those without SR, were more frequently women (19% vs 10%), 27% vs 28% used EFV, and had 2.17 (SD:1.39) assessments/year vs 1.62 (SD:0.84). SR rates per 1000 patients among those who were evaluated, increased significantly from 0.03 in 2018, 0.26 in 2019, 3.14 in 2020 and 10.58 in 2021. Throughout the model, independently of other covariates, no significant changes in SR were observed during 2019 and 2020, compared to 2018: OR 1.21 (0.57-2.52), p=0.61 and OR 2.01 (0.55-1.00), p=0.06; but we found a significant increase in the SR in 2021: OR 3.71 (1.55-8.88), p=0.003. In this model, EFV use vs non-EFV use was not associated with SR: OR 0.78 (0.39-1.56), p=0.48.

Conclusion: After an adjusted analysis, suicidal risk in PWH was significantly higher after the lock-down due to COVID19, independently of EFV use. These results should encourage HIV health providers to actively look for suicidal risk and incorporate specialized mental care.

Significance of Elevated SARS-CoV-2 Antigen Levels During Early Hospitalization
Mamta K. Jain
ACTIV-3/TICO Study Group
University of Texas Southwestern, Dallas, TX, USA

Background: Patients hospitalized with COVID-19 randomized to standard of care (SoC) plus placebo or SoC plus monoclonal antibody (mAb)[bamlanivimab, sotrovimab, ambrolvirb-romlusevimab, or tixagavimab-cilagavimab] as separate arms of TICO/ACTIV-3 did not show differences in the time to sustained recovery through day 90. Combining these cohorts, we assessed if early changes in plasma nucleocapsid antigen (pNA) were associated with clinical outcomes.

Methods: TICO/ACTIV-3 enrolled 2,254 patients between 8/5/2020 to 9/30/2021. We used the Quanterix assay to measure pNA of stored samples. We selected those with pNA in the top quartile at baseline through day 5 and the bottom quartile at day 5 as high and low, respectively. We used chi-square test to compare SR rates among those with high and low pNA at baseline through day 5. We used the Quanterix assay to measure pNA at baseline through day 5 and the day 5 to day 90 wk1 and wk2 period of SR. We used the Quanterix assay to measure pNA at baseline through day 5 and the day 5 to day 90 wk1 and wk2 period of SR. We used the Quanterix assay to measure pNA at baseline through day 5 and the day 5 to day 90 wk1 and wk2 period of SR.
**IMMEDIATE AND CONTRIBUTORY CAUSES OF DEATH IN PATIENTS HOSPITALIZED WITH COVID-19**

Adeel Butt1, Mylai D. Guerrero2, Eleonor B. Cañas1, Husni Al-Diwai2, Aseel Hatem Subhi Alzibdeh1, Thasneem Odaippurath2, Ali Ahmed Sheikh Saleh2, Muna Al-Maslamani3, Abdul-Badi Abou-Samra3, Giuseppe Lapadula1, Luca Mezzadri1, Giustina Lo Cascio1, Sergio Malandrin2, Alice Ranzani2, Silvia Limonta2, Annalisa Cavallerio2, Paolo Bonfanti3, Mylai D. Guerrero2, Elenor B. Canlas2, Husni Al-Dwairi2, Aseel Butt1, Mylai D. Guerrero2, Eleonor B. Cañas1, Husni Al-Diwai2, Aseel Hatem Subhi Alzibdeh1, Thasneem Odaippurath2, Ali Ahmed Sheikh Saleh2, Muna Al-Maslamani3, Abdul-Badi Abou-Samra3, Giuseppe Lapadula1, Luca Mezzadri1, Giustina Lo Cascio1, Sergio Malandrin2, Alice Ranzani2, Silvia Limonta2, Annalisa Cavallerio2, Paolo Bonfanti3, Mylai D. Guerrero2, Elenor B. Canlas2, Husni Al-Dwairi2, Aseel Butt1, Mylai D. Guerrero2, Eleonor B. Cañas1, Husni Al-Diwai2, Aseel Hatem Subhi Alzibdeh1, Thasneem Odaippurath2, Ali Ahmed Sheikh Saleh2, Muna Al-Maslamani3, Abdul-Badi Abou-Samra3, Giuseppe Lapadula1, Luca Mezzadri1, Giustina Lo Cascio1, Sergio Malandrin2, Alice Ranzani2, Silvia Limonta2, Annalisa Cavallerio2, Paolo Bonfanti3

**Background:** Accurate determination of the immediate and contributory causes of death in patients with COVID-19 is important for optimal care and instituting mitigation strategies.

**Methods:** All deaths in Qatar between March 1, 2020 and August 31, 2022 flagged for likely relationship to COVID-19 by were evaluated by two independent reviewers trained to determine and assign the most likely immediate underlying cause of death. Each decedent’s electronic medical records was comprehensively reviewed, and the cause of death was assigned based on the most plausible underlying event that triggered the event(s) that led to death based on clinical documentation and a review of laboratory, microbiology, pathology, and radiology data. After cause assignment, each case was categorized into major diagnostic groups by organ system, syndrome, or disease classification.

**Results:** Among 749 deaths flagged for likely association with COVID-19, the most common admitting diagnoses were respiratory tract infection (91%) and major adverse cardiac event (MACE, 2.3%). The most common immediate cause of death was COVID pneumonia (66.2%), followed by MACE (7.1%), hospital associated pneumonia (HAP, 6.8%), bacteremia (6.3%), disseminated fungal infection (DFI, 5.2%), and thromboembolism (4.5%). The median length of hospital stay was 23 days (IQR 14,38). COVID pneumonia remained the predominant cause irrespective of the time from admission, though the proportion dropped with increasing length of stay in the hospital. Other than COVID pneumonia, MACE was the predominant cause of death in first two weeks but declined thereafter. No death occurred due to bacteremia, HAP, or DFI in the first week after hospitalization, but became increasing common with increased length of stay in the hospital accounting for 9%, 12%, and 10% of all deaths after 4 weeks in the hospital respectively. The majority of deaths (86%) occurred in the intensive care unit setting. COVID pneumonia accounted for approximately two-thirds of deaths in each setting. MACE and HAP were approximately equally represented in both settings while bacteremia and disseminated fungal infection were more common in the intensive care unit setting.

**Conclusion:** Nearly one-third of patients with COVID infection die of non-COVID causes, some of which are preventable. Mitigation strategies should be instituted to reduce the risk of such deaths.

**Figure 1. Immediate cause of death by time of death after hospital admission.**

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**ANTI-S-RBD TITER AND RISK OF CLINICAL PROGRESSION IN PATIENTS WITH COVID-19 PNEUMONIA**

Giuseppe Lapadula1, Luca Mezzadri1, Giustina Lo Cascio1, Sergio Malandrin2, Alice Ranzani2, Silvia Limonta2, Annalisa Cavallerio2, Paolo Bonfanti3

1University of Milano-Bicocca, Monza, Italy, 2Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy

**Background:** Antibodies (Ab) against the receptor-binding-domain of the spike protein (anti-S-RBD) elicited by SARS-CoV-2 infection or vaccination are deemed to be a correlate of protection. We aimed at assessing whether anti-S-RBD titer is associated with the outcome of subjects hospitalised with COVID-related pneumonia.

**Methods:** Adults hospitalized between Jul 2021 and Jul 2022 for COVID-19 with respiratory failure (SpO2 < 93% on room air) or radiological evidence of pneumonia were included if anti-S-RBD titer was measured within 72 h of admission. Time between admission and death/need for intubation was described using Kaplan-Meier curves. Cox Regression analysis, stratified for vaccination status, was used to explore the association between anti-S-RBD titer and survival. Age, gender, days since symptom onset immunosuppressive conditions and use of monoclonal Ab (mAb) were explored as possible confounders.

**Results:** 534 patients were enrolled. Their mean age was 71 years, 63% were male and 51% vaccinated; 42% had ≥1 immunosuppressive condition among hematology or solid malignancy, HIV, diabetes, end-stage renal failure, liver cirrhosis, organ transplant or immunosuppressive treatment. Antibody titer was significantly higher among vaccinated than among unvaccinated patients (1366 vs 158 BAU/mL; p < 0.001). Among vaccinated subjects, lower titer of anti-S-RBD were measured among those with hematomal malignancies (1282 vs 471 BAU/mL; p < 0.001) or who were receiving immunosuppressive therapy (1287 vs 537 BAU/mL; p < 0.001).

Older age, shorter time between onset of symptoms and hospitalization and immunosuppressive conditions were associated with higher rates of death or intubation (Fig 1). Using Cox regression stratified for vaccination, a significant association between anti-S-RBD titer and risk of death/intubation was observed (per log2 BAU/mL increase, HR 0.90, 95%CI 0.88–0.95; p = 0.020), independently of age (per year increase, HR 1.03; 95%CI 1.01–1.04), male gender (HR 1.00; 95%CI 0.70–1.42) and presence of immunosuppressive conditions (HR 1.46; 95%CI 1.01–2.10).

Adjustment for mAb treatment did not change the results to a significant extent.
Conclusion: Low anti-S-RBD titer was associated with poor outcome among patients hospitalized for COVID-19-related pneumonia, regardless of vaccination. In addition, older age and presence of immunosuppressive conditions remain important predictors of mortality. Kaplan-Meier Curves for intubation-free survival according to age, days from symptoms’ onset, presence of immunosuppressive conditions and anti-S-RBD titer.

731 AI-BASED PREDICTION OF LUNG TISSUE INVOLVEMENT IS PREDICTIVE OF COVID-19 SEVERITY
Miłosz Parczewski, Bogusz Aksak-Wąs, Daniel Obher, Laura Leśiewska, Malwina Karasińska-Cieślak, Krystian Awgul, Adam Majchrzak, Karol Serwin Pomeranian Medical University, Szczecin, Poland

Background: During COVID-19 epidemics several artificial-intelligence neural networks (ANN) systems were developed classify the risk of disease progression to respiratory failure and death, providing aid for clinical decision. However, for optimal results these models should link multiple medical data in a simple model. In this study we analyse the in-hospital mortality and mechanical ventilation risk using combination ANN based rapid computed tomography assessment tool and selected clinical variables.

Methods: Data of 4317 COVID-19 hospitalized patients including 266 cases required mechanical ventilation were analysed using newly constructed and locally trained ANN algorithm. Demographic, clinical, laboratory, and ANN-based lung inflammation data were analysed using proportional Cox Hazards model and estimate in-hospital mortality and intensive care admission risk.

Results: Overall in-hospital mortality associated with ANN-assigned percentage of the lung involvement (HR 5.72 (95%CI: 4.4-7.43), p< 0.001 for the patients with >50% of lung tissue affected by COVID-19 pneumonia, age category (HR 5.34 (95%CI: 3.32-8.59) for cases >80 years, p< 0.001), procalcitonin > 2 (HR: 2.1 (95%CI: 1.59-2.76) ng/ml p< 0.001, C-reactive protein level category (max HR 2.11 (95%CI: 1.25-3.56) for CRP >100 mg/dL, p=0.004), estimated glomerular filtration rate (max HR 1.82 (95%CI: 1.37-2.42), p< 0.001 for eGFR < 30 ml/min) and troponin increase above upper limit normal level (HR: 2.14 (95%: 1.69-2.72), p< 0.001) (Figure 1). Furthermore, risk of mechanical ventilation also associated with ANN-based percentage of lung inflammation (HR 13.2 (8.65-20.4), p< 0.001 for patients with >50% involvement), age, procalcitonin > 2 mg/ml (HR: 1.91 (95%CI: 1.14-3.2), p=0.14 estimated glomerular filtration rate (HR 1.82 (1.2-7.4), p=0.004 for eGFR < 30 ml/min) but also clinical variables, including (HR: 2.5 (95%CI: 1.91-3.27), p< 0.001), cardiovascular and cerebrovascular disease (HR: 3.16 (95%CI: 2.38-4.2), p< 0.001), and chronic pulmonary disease (HR: 2.31 (95%CI: 1.44-3.7), p< 0.001).

Conclusion: ANN-based lung tissue involvement was the strongest predictor of unfavorable outcomes in COVID-19, and represent valuable support tools for clinical decisions.

Adjusted multivariate model for the risk of COVID-19 in-hospital mortality

<table>
<thead>
<tr>
<th>AI-based percentage of lung involvement (%)</th>
<th>HR (95%CI; p=value)</th>
</tr>
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<tbody>
<tr>
<td>50-55</td>
<td>2.7 (1.3-5.4; 0.01)</td>
</tr>
<tr>
<td>70-90</td>
<td>4.2 (2.4-7.1; 1e-05)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>6.0 (3.3-11; 2e-05)</td>
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</table>

Adjusted Cox mortality for all hospitalized patients:

<table>
<thead>
<tr>
<th>Titer</th>
<th>HR (95%CI; p=value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>0.6 (0.4-0.9; 0.01)</td>
</tr>
<tr>
<td>0.005</td>
<td>0.4 (0.3-0.7; 0.001)</td>
</tr>
</tbody>
</table>

732 A JOINT ANALYSIS OF TWO RANDOMIZED CONTROLLED TRIALS ON Enoxaparin for COVID-19
Giovanni Dolci1, Cinzia Del Giovanni1, Massimo Arquati2, Valeria Coluccio1, Erica Franceschini1, Roberto D’Amico3, Roberto Vicini3, Pasquale Miglioli1, Riccardo Colombo4, Alba Taino5, Pietro Facchinetti4, Lucia Trombetta4, Francesca Tonelli4, Marco Marietta4, Maddalena Alessandra Wu6
1University of Modena and Reggio Emilia, Modena, Italy, 2Columbus Center Clinic, Milano, Italy, 3Istituto Ospedaliero Universitario Policlinico di Modena, Modena, Italy, 4Fatebenefratelli Sacco Hospital, Milano, Italy, 5FATEBENEFRAHITI, Milano, Italy

Background: COVID-19 carries a high risk of vascular thrombosis. This joint analysis of two randomized-controlled trials (RCTs) aims to assess the safety and efficacy of enoxaparin at therapeutic dose compared to prophylactic dose in people hospitalized with COVID-19.

Methods: A joint analysis of two RCTs, COVID-19 HD (NCT044082359) and EMOS-COVID (NCT04646655), was performed. Both studies enrolled inpatients with COVID-19- associated respiratory compromise (as identified by respiratory rate >25 breaths/min or arterial oxygen saturation ≤93% at rest or PaO2/FIO2 ≤200 mmHg for COVID-19 HD and by PaO2/FIO2 ≤250 mmHg for EMOS-COVID) and/or coagulopathy (D-dimer > 2000 ng/ml for both RCTs or sepsis-Induced coagulopathy score > 4 for COVID-HD). In both RCTs patients were randomly assigned to two arms: enoxaparin at prophylactic dose (standard 4 000 IU; in the EMOS-COVID 6000 IU if body weight >100 kg) and at therapeutic dose (70 U/Kg every 12 h).

The primary efficacy endpoint of the joint analysis was clinical worsening, defined as the occurrence of at least one among: in-hospital death; acute myocardial infarction; symptomatic arterial or venous thromboembolism; need of either Continuous Positive Airway Pressure (Cpap) or Non-Invasive Ventilation (NIV) in patients who were in standard oxygen therapy at randomization; need for IMV in any patient. The primary outcome was assessed as time-to-event, described with hazard ratio (HR) and with Kaplan-Meier survival estimate. The primary safety endpoint was major bleeding for both trials and for the joint analysis.

Results: COVID-19 HD enrolled 142 people between July 2, 2020 and February 15, 2022, while EMOS-COVID enrolled 141 people from July 27, 2020 to June 5, 2021, resulting in 283 patients included in this joint analysis. Two-hundred-seven (73.1%) were males, with a mean age of 61.1 years (SD ±10.7), a mean BMI of 29.7 kg/m2 (SD ±5.0), and 115 (40.6%) were on NIV or Cpap at randomization, seven (73.1%) were males, with a mean age of 61.1 years (SD ±10.7), a mean BMI of 29.7 kg/m2 (SD ±5.0), and 115 (40.6%) were on NIV or Cpap at randomization.

No major bleeding was observed during the study time.

Conclusion: The results of this joint analysis did not highlight significant differences in clinical worsening between COVID-19 patients that received enoxaparin at therapeutic dose compared to prophylactic dose.
THE IMPACT OF EARLY OUTPATIENT TREATMENTS FOR COVID-19: A RETROSPECTIVE STUDY

Silvia Amadasi1, Paola Bertuccio1, Melodia Degli Antoni2, Francesca Viola1, Davide Minisci1, Francesco Rossini1, Alex Francesco Bolandrini1, Francesco Castelli1, Anna Odone2, Eugenia Quiros-Roldan1

1University of Brescia, Brescia, Italy, 2University of Pavia, Pavia, Italy

Background: Oral antivirals (nirmatrelvir/ritonavir and molnupiravir), intravenous short treatment of remdesivir and anti-SARS-CoV-2 monoclonal antibodies (mAbs) have been used for early COVID-19 treatments in high risk of disease progression patients. Little is known about the impact of therapies on post-acute COVID-19 (PACS). We aimed to compare the efficacy of these therapies in terms of death, hospitalization rate and PACS at 3 months.

Methods: We conducted a retrospective observational study including all eligible outpatients aged ≥18 evaluated from April 2021 to March 2022 at our COVID-19 Clinic. Patients were stratified into 3 groups: mAbs, antivirals (oral and short-course remdesivir) and controls (eligible patients who refused treatment). Persistence of symptoms (fever, dyspnea/anosmia, cough, pharyngodynia, dyspnea, chills, nasal congestion, myalgia, headache, gastrointestinal disease, and neuro-behavioural symptoms, such as asthenia, anxiety/mood disorder, memory and concentration deficit) were evaluated after 3 months. We estimated the associations between each considered outcome and treatment through univariable and multivariable logistic models adjusted by sex, age, vaccination, early COVID treatment, treatment group and number of comorbidities (when appropriate).

Results: We included 649 patients (51.6% males, median age 67 years, 14% unvaccinated): 242 patients were treated with mAbs, 197 with antivirals and 210 received no treatment. Overall, 36.7% of subjects had cerebro-cardiovascular disease, 22% were obese and 50% had more than one comorbidity. Overall, 29 patients (4.5%) died or were hospitalized. Death or hospitalization was positively associated only with older ages with a significant linear trend (p for trend: 0.033). Data on PACS at 3 months were available for 323 (49.8%) patients. Females showed a positive association with long COVID, with an OR of 2.14 (95% CI: 1.30-3.53) as compared to men. Patients treated with antiviral drugs showed an inverse association with long COVID (OR: 0.43, 95% CI: 0.21-0.87 as compared to not treated patients). Patients who were treated with monoclonal antibodies showed an OR of 0.48 (95% CI: 0.25-0.92) as compared to those in the control group (Table 1).

Conclusion: The impact of early COVID-19 therapies on PACS is unknown. Our results showed that these treatments, in particular mAbs, can reduce persistence of neuro-behavioural symptoms at 3 months.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Numbers and percentages of “long COVID” according to selected covariates and results from the logistic regression models</th>
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<tbody>
<tr>
<td>Associated with outcome</td>
<td>Treatment (N=649)</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
</tr>
<tr>
<td>Death</td>
<td>11 (4.5%)</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>119 (36.7%)</td>
</tr>
<tr>
<td>Long COVID</td>
<td>141 (43.7%)</td>
</tr>
</tbody>
</table>

CLINICAL PREDICTORS OF MPOX SEVERITY IN AN ITALIAN MULTICENTER COHORT (MPOX-ICONA)

Valentina Mazzotta1, Alessandro Tavelli2, Francesca Colavita1, Lorenzo Biasioli1, Antonella Castagna1, Annalisa Mondi2, Fabrizio Carletti1, Davide Moschese1, Luca Pipito1, Daniele Tesor1, Roberta Gagliardini1, Fabrizio Maggi1, Andrea Antonini1, Antonella D’Arminio Monforte1, Simone Lanini2

1National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy, 2Icona Foundation, Milan, Italy

Background: Mpox is a pivotal example of how old viral pathogens can emerge as the cause of new outbreaks with novel clinical presentation and topical route of transmission. Here we present an analysis to explore potential predictors of Mpox severity and viral persistence in relevant biological fluids.

Methods: Italian multicenter cohort study. Severe cases were those who needed hospitalization or had proctitis, pharyngotonsillitis, ocular lesions, or >20 skin lesions. Recovery was defined as the resolution of all mucocutaneous lesions. Bivariable analysis of severity predictors was carried out with X2 test. Logistic regression model was used for multivariate analysis. The analysis to assess early viral load (VL) as predictor of severity was done by Student T-Test and logistic regression model.

Results: Between 16 May and 31 Oct 2022, 202 pts were enrolled; they were all male with a mean age of 38 years (range 21-75). Mean duration of disease was 22.4 days (95%CI 20.93-23.34). Multivariable analysis to explore severity predictors (Fig1A) showed that fever (p=0.001), facial lesions (p=0.045), anal lesions (p=0.013), and concurrent STIs (p=0.032) were associated with severe Mpox. Quantitative determination of VL load in the upper respiratory tract (propharyngeal swab or saliva, URT) was available for 79 pts (39 mild and 40 severe). Mean Ct-value was 31.91 (95%CI 29.29-34.54) and 26.68 (95%CI 24.68-28.68) in mild and severe cases, respectively (P< 0.002; Fig1B). Logistic regression model was used for multivariate analysis. The analysis to assess early viral load (VL) as predictor of severity was done by Student T-Test and logistic regression model.

Conclusion: We found that pts presenting with fever, facial/anal lesions, and concurrent STIs may develop more severe Mpox. Moreover, higher VL in URT during the first week after symptoms onset was associated with severe disease. Our findings may serve to guide management of pts with Mpox in terms of need for hospitalization and drug therapy. Finally, our study claims an urgent need to assess whether the persistence of MPXV in biological samples after clinical recovery may lead to a status of persistent infectivity.

Figure legend. A) Analysis of risk for severe disease (N=202 patients). Left columns descriptive analysis. Middle columns bivariable analysis of risk (p-values are reported according to X2 test). Right columns adjusted analysis according to multivariable logistic model (P-values are reported according to Wald’s test). B) Observed Ct-value in upper respiratory tract during the first week of disease (N=79 patients; P-values are reported according to Student T-test). C) Association between severe disease and Ct-values in the upper respiratory tract during the first week of disease (N=79 patients; P-values are
SEVERE MPX IN NEW YORK CITY

Elizabeth A. Garcia 1, Maura K. Lash 1, Tristan D. McPherson 1, Mary Foote 1, Karen A. Alroy 1, Ellen H. Lee 1, Wendy Wen 1, Jeanne Sullivan Meissner 1, Amma Bosompem 2, Alyssa Boascur 1, Marcia Wong 1, Victoria Tittle 1, Anton L. Pozniak 1, Marta Boffito 1

1New York City Department of Health and Mental Hygiene (DOHMH) and 2New York City Department of Social Services (DSS) for DSS housing status.

Background: The mpox clinical course among people with HIV (PWH) during the 2022 outbreak is poorly understood. We describe mpox among PWH in New York City (NYC) who received > 14 days of tecovirimat and/or coadministration of additional mpox treatments.

Methods: We identified PWH with persistent or worsening mpox during tecovirimat treatment through healthcare providers who called NYC Department of Health and Mental Hygiene (DOHMH) or Centers for Disease Control and Prevention's clinical team for consultation between 8/2/2022 and 12/16/2022. We collected demographics and HIV/mpox clinical information. We crossmatched cases with DOHMH HIV surveillance for CD4 and viral load (VL), Citywide Immunization Registry for Jynneos vaccination, and NYC Department of Social Services (DSS) for DSS housing status.

Results: We identified 11 cases. Median age was 38 years (range: 29–45); 9 (73%) were Black non-Hispanic and 2 (18%) were Hispanic. Six (50%) had a recent history of being unstably housed; with 4 (36%) unstably housed at mpox diagnosis. Nine (82%) had a mpox diagnosis. Median HIV VL was 237,000 copies/mL (range: 73,000–2 million). All had CD4 < 200 cells/mm 3 ; 8 (73%) had CD4 < 50 cells/mm 3 . Three (27%) received one dose of tecovirimat; (8% had mpox in all settings with 5 days of vaccine. Median time from initial specimen collection to tecovirimat initiation was 5.5 days (range: 0-16, data available for 10 cases). Tecovirimat initiation was 5.5 days (range: 0-16; 12/16, 1 case (9%) had lesion resolution, 5 (45%) were still hospitalized on 12/16/2022. Other medications coadministered with tecovirimat included vaccinia immune globulin (VIG) (n=9, 82%), cidofovir, brincidofovir and foscarnet (each with n=2, 18%). Of cases receiving VIG, 5 (56%) received multiple doses. All 11 PWH were hospitalized with severe mpox manifestations. Examples included necrotic facial lesions, severe eye involvement and airway edema, and uncontrollable rectal bleeding. As of 12/16/2022, 1 case (9%) had lesion resolution, 5 (45%) were still hospitalized on treatment, 4 (36%) died, and 1 (9%) had unknown clinical disposition.

Conclusion: This group of PWH with advanced HIV had severe mpox manifestations and poor response to tecovirimat. Early and extended tecovirimat with coadministration of other mpox treatments in the setting of limited options is important to try to improve outcomes. Findings of severe disease and high mortality highlight the urgency of mitigating deep social inequities and high-quality research to optimize care in this group of PWH.

MANAGEMENT OF MPX IN PWH ATTENDING A SEXUAL HEALTH DEPARTMENT IN LONDON, UK

Nicolò Girometti, Felicity Burton, Matthew Spencer, Simisola Agunbiade, Ruth Byrne, Margherita Bracci, Rachael Jones, Ellen Dwyer, Gary G. Whitlock, Victoria Tittle, Anton L. Pozniak, Marta Boffito

Chelsea and Westminster Hospital, London, United Kingdom

Background: MPX has disproportionately affected people with HIV (PWH) since its global spread and preliminary data suggest a higher burden of complications and worse outcomes in this group.

Methods: Aggregated and anonymised data on clinical characteristics, medical management and concurrent STIs rates in PWH with PCR-confirmed MPX infection between May 15th- Dec 15th 2022 attending a large, UK sexual health service were retrospectively collected from electronic patient records. Risk factors for clinical complications and hospital admission were extrapolated using Fisher’s exact test.

Results: 249 PWH were included (28.5% of the 873 total MPX diagnoses) (Table 1), median age 39 years (IQR 33-47), 98% MSM, 74% white ethnicity. Four individuals were newly diagnosed with HIV at time of MPX presentation, with 5% having a HIV viral load >200 copies/mL and 95% being on cART. Ten individuals had a CD4 count < 350/mm 3 with an overall median CD4 count of 697/mm 3 (IQR 544-897). Prodromal symptoms were common (fever 59%, fatigue 41%, myalgia 30%, sore throat 16%) and whilst 3% did not report any skin lesions, 10% presented with at least one skin lesion, 62% with 2-10 lesions and 24% over 10 lesions. Skin involvement had a predominantly perianal (52%), limb (45%) and genital (38%) distribution, with only a fifth of cases without perianal or the genital involvement. Complications occurred in 43% of cases, with perianal pain (26%), proctitis (14%), bacterial superinfection/cellulitis (12%), constipation (8%), penile oedema (4%) and tonsillitis (2%) being the most common. Additional medical management was often required (43%), with analgesia (32%), antibiotic therapy (26%) or laxatives (8%) prescribed most frequently. Eight individuals (3%) required hospital admission, with no fatal outcomes. The presence of anal lesions and/or a CD4 count < 350/mm 3 were associated with a higher burden of medical complications (p=0.02), with the latter also associating with hospitalization (p=0.05). A total of 31% individuals had a concurrent STI at time of MPX presentation: 18% had gonorrhoea (67% were rectal infections), 15% chlamydia (81% were rectal infections) and 8% syphilis.

Conclusion: Despite low hospitalization rates in PWH with MPX, medical complications and STIs rates requiring further management are significantly high. Further comparative analysis with people without HIV and PWH with severe immunodeficiency are needed to define risk factors for hospitalization and clinical complications.

Table 1. Demographics and clinical characteristics of PWH with PCR-confirmed MPX attending the sexual health clinics at Chelsea and Westminster Hospital NHS Foundation Trust (London, UK).

<table>
<thead>
<tr>
<th>MPXdiagnosed with PWH (n=249)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>Med age, years (IQR)</td>
<td>38 (23-47)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>122 (50)</td>
</tr>
<tr>
<td>Day, illness onset among men</td>
<td>249 (99)</td>
</tr>
<tr>
<td>Transgender</td>
<td>5 (2)</td>
</tr>
<tr>
<td>White ethnicity</td>
<td>272 (11)</td>
</tr>
<tr>
<td>Black ethnicity</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Black Brit / Afro-Caribbean</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Mean number of sex partners within 90 days prior MPX, (IQR)</td>
<td>3.0 (2-4)</td>
</tr>
<tr>
<td>CART history</td>
<td></td>
</tr>
<tr>
<td>On ART at time of MPX diagnosis</td>
<td>212 (86)</td>
</tr>
<tr>
<td>Latest CD4 count (cells/mL)</td>
<td>507 (544-597)</td>
</tr>
<tr>
<td>Latest CD4 cell count (cells/mm 3)</td>
<td>10 (3-16)</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
</tr>
<tr>
<td>Fever, headache, fatigue, myalgia</td>
<td>241 (92)</td>
</tr>
<tr>
<td>Cold, cough, rhinitis, sore throat</td>
<td>101 (40)</td>
</tr>
<tr>
<td>Back pain</td>
<td>57 (23)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Proctitis</td>
<td>6 (2)</td>
</tr>
<tr>
<td>No prodromal symptoms</td>
<td>66 (27)</td>
</tr>
<tr>
<td>Prodromal symptoms presenting after onset of skin lesions</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Skin lesions localization</td>
<td></td>
</tr>
<tr>
<td>Perianal</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Genital</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Limb</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Head</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Number of skin lesions</td>
<td></td>
</tr>
<tr>
<td>No skin lesions</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Single lesion</td>
<td>20 (8)</td>
</tr>
<tr>
<td>2-5 lesions</td>
<td>150 (60)</td>
</tr>
<tr>
<td>6-10 lesions</td>
<td>51 (20)</td>
</tr>
<tr>
<td>&gt; 10 lesions</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Inginal lymphadenopathy</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Oral mucosal involvement</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Clinical complications</td>
<td></td>
</tr>
<tr>
<td>Anal pain</td>
<td>66 (26)</td>
</tr>
<tr>
<td>Proctitis</td>
<td>58 (23)</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>20 (8)</td>
</tr>
<tr>
<td>Tetanus</td>
<td>20 (8)</td>
</tr>
<tr>
<td>Pertussis</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Tetanus</td>
<td>6 (2)</td>
</tr>
</tbody>
</table>

Outcome disposition by date of first symptom.
**Development and Pilot of an Mpox Severity Scoring System (Mpox-SSS)**

Jason Zucker1, Jacob McLean, Simian Huang, Clare Delaurentis, Shauna Gunaratne, Kate Stockley, Marshall J. Glesby, Timothy Wilkin, William Fischer, Inger Damon2, John Brooks3

1Columbia University Medical Center, New York, NY, USA, 2Columbia University Irving Medical Center, New York, NY, USA, 3Columbia University, New York, NY, USA, 4Wellcome Centre, The University of Edinburgh, Edinburgh, UK, 5University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 6Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Clinical severity scores facilitate standardized quantitative comparison of disease severity between groups of patients to understand risk factors for severe illness and evaluate treatment efficacy. Relevant severity scoring tools provide reliable discrimination across the spectrum of illness severity and are parsimonious, easy to use, and universally applicable. Prior mpox severity scores, based on numbers of skin lesions and individual functional capacity, have been less applicable to the 2022 outbreak.

**Methods:** Using expert opinion and literature review, we developed an MPOX-SSS with an initial set of possible variables that we refined based on data availability, prior association with severity, and parameter correlation to include 7 final elements: number of active lesions, anatomic extent of lesion involvement, presence of confluent lesions, presence of bacterial superinfection, extent of mucosal areas affected, level of care, and analgesia requirement (tool available at mpoxseverityscore.com). We piloted this MPOX-SSS via a retrospective chart review at a single academic urban medical center and compared scores using the Wilcoxon rank sum test.

**Results:** Among the first 200 patients presenting with mpox (median age 34, 99% born male, 38% Hispanic, 28% Black, 49% with HIV [10% CD4 count < 200 cells/mm3 or VL > 1000 copies/mL], 57% treated with tecovirimat), an MPOX-SSS score could be calculated for 86%; missing data that precluded scoring included lesion number (13%) and presence of confluent lesions (7%). Median scores were similar in patients with and without HIV (8 vs 9, p=0.12) (Figure 1A). Scores were higher in patients treated with tecovirimat (10 vs 4, p<0.001). Patients with CD4 counts < 200 cells/mm3 (10 vs 8, p=0.073), and patients presenting >3 days after symptom onset (9 vs 6, p=0.007). For a subset of individuals with multiple visits for mpox, changes in MPOX-SSS scores were detected and concordant with clinical experience (Figure 1B).

**Conclusion:** Our pilot MPOX-SSS was able to produce a severity score retrospectively from 86% of charts, demonstrated good discrimination with statistically higher scores in groups expected to have more severe disease, and was able to distinguish change over time for individual patients that correlated with clinical illness. We propose this tool be assessed for utility in clinical trials of mpox treatment, in prospective observational cohort studies, and in comparisons of illness caused by different mpox clades.

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**Clinical presentation of mpox in people with and without HIV**

Victoria Pilkington1, Kilian Quinn2, Lucy Campbell3, Michael Brady4, Frank Post1

1King’s College HIV and Sexual Health Research Group, 2King’s College London, London, United Kingdom, 3King’s College Hospital, London, United Kingdom, 4King’s College Hospital NHS Foundation Trust, London, United Kingdom

**Background:** Early UK surveillance data revealed that people living with HIV (PLWH) were overrepresented within monkeypox (mpox), with one third of mpox cases reported in PLWH. However, it is unknown whether mpox infection is more severe in people living with HIV.

**Methods:** All laboratory confirmed mpox cases seen between May–December 2022 in one London hospital trust were identified via a search of pathology reporting systems. Under existing clinical pathways, patients received regular telephone reviews (virtual ward) until deemed safe to discharge. We
extracted demographic and clinical data, including length of follow up and hospitalisations to allow comparison of HIV positive and negative cases. Data were analysed using STATA 17.

**Results:** 150 cases of mpox were identified (mean age 37.4, 99.3% male, 92.7% MSM), 58 (38.7%) of whom were in PLWH (mean CD4 cell count 513 cells/mm\(^3\)) and 47 (31.0%) with HIV RNA < 200 copies/mL. HIV positive mpox cases were older (40.4 vs 35.4years, p=0.001) but otherwise similar to those without HIV. Compared with HIV negative mpox cases, HIV positive cases had similar clinical presentations with similar risk of more widespread manifestations of disease, such as non-dermatological symptoms (87.9% vs 82.6%, p=0.38) and extra-genital lesions (74.1% vs 64.0%, p=0.199). PLWH experienced a similar time to from onset of symptoms to discharge (mean days 17.1 vs 15.4, p=0.39) and total time under virtual ward review (mean days 11.7 v 9.01, p=0.13). A similar proportion of people with HIV required review in the emergency department (36.2% vs 25.6%, RRR=1.45, 95%CI=0.86 to 2.33). A higher proportion of PLWH were admitted to hospital, but this did not reach statistical significance (19.0% vs. 9.30%, RRR=2.04, 95%CI=0.86 to 4.76). Of the small sample of PLWH with uncontrollable viral loads (RNA > =200), 2/5 patients (40%) were hospitalised. There were no recorded deaths.

**Conclusion:** In this cohort of mpox cases there was a high prevalence of well-controlled HIV co-infection, but we find no evidence that PLWH experience more severe mpox. Whilst there is a higher proportion of hospitalisations, this is not statistically significant and is likely to be impacted by additional caution shown by clinicians in making decisions around mpox care in these patients. All other outcomes analysed indicate that mpox infections are of similar severity in people with and without HIV, providing reassurance for patients and clinicians providing future care for patients with mpox and HIV co-infection.

### 740 TB OUTCOMES IN PEOPLE LIVING WITH HIV: AN INTEGRATIVE DATA ANALYSIS OF PHASE 3 TRIALS

**Rob van Wijk,** Vincent Chang, Marjorie Imperial, Patrick Phillips, Susan Swindells, Richard Chaissone, Payam Nahid, Rodney Dawson, Ian Sanne, Ziyaad Waja, Susan Dorman, Ekaterina Kurbatova, Rada Savic, Rob van Wijk

**Background:** People with HIV (PWH) and tuberculosis (TB) have been historically at higher risk of TB-related unfavorable outcomes than those without HIV. Our goal is to understand risk factors for TB-related outcomes in PWH, by performing an integrative data analysis of PWH in 4 recent Phase 3 TB trials.

**Methods:** Integrative analysis included data from PWH in 4 recent Phase 3 TB treatment trials (OFLOTUB, RIFAQUIN, S31, and RIFAQUIN EMRZ/2P2M2). Cox proportional hazard of the integrated data analysis of four Phase 3 clinical trials showing hazard ratios (HR) with 95% confidence intervals. Cox proportional hazard of the integrated data analysis of four Phase 3 clinical trials showing hazard ratios (HR) with 95% confidence intervals.

**Results:** The dataset included 711 (11.2% of dataset) PWH (of which 45.4% female). Median (range) baseline CD4 count was 339 (19-1155) cells/mm\(^3\) and 359 (50.5%) were not on ART. Ninety-three unfavorable events occurred. RIFAQUIN’s 6-month moxifloxacin-, and S31’s 4-month rifapentine-moxifloxacin regimens performed similarly to standard-of-care (Figure 1). ART use (89% efavirenz-based) correlated with trial (only S31 and RIFAQUIN allowed ART); however PWH receiving ART (71%) had fewer TB-related unfavorable outcomes compared to PWH not receiving ART (18.9%). Multivariate analysis showed that male PWH (adjusted hazard ratio [HR] with 95% confidence interval 2.08 (1.34-3.24) compared to female) had high baseline smear grade (HR 1.25 (0.62-2.53) and 2.27 (1.26-4.09) for 2+ and 3+ respectively, compared to negative or 1+), and with decreased adherence (HR 1.04 (1.01-1.07) per percent point decrease) were predictive of unfavorable outcomes. Male PWH with smear grade 2+ or 3+ had rates of TB-related unfavorable outcomes (12.9% with ART and 25.8% without ART) compared to patients at lower risk (defined as female PWH or male PWH with negative smear grade or 1+) (7.1% with ART and 13.5% without ART). Conclusion: PWH are efficaciously treated with anti-TB regimens containing rifapentine and moxifloxacin. Male PWH with baseline smear grade 2+ or 3+ and lower treatment adherence are at highest risk of unfavorable TB outcome. Positive effects of ART while undergoing TB treatment are confirmed.

**Conclusion:** There were no recorded deaths.

**Results:** 150 cases of mpox were identified (mean age 37.4, 99.3% male, 92.7% MSM), 58 (38.7%) of whom were in PLWH (mean CD4 cell count 513 cells/mm\(^3\)) and 47 (31.0%) with HIV RNA < 200 copies/mL. HIV positive mpox cases were older (40.4 vs 35.4years, p=0.001) but otherwise similar to those without HIV. Compared with HIV negative mpox cases, HIV positive cases had similar clinical presentations with similar risk of more widespread manifestations of disease, such as non-dermatological symptoms (87.9% vs 82.6%, p=0.38) and extra-genital lesions (74.1% vs 64.0%, p=0.199). PLWH experienced a similar time to from onset of symptoms to discharge (mean days 17.1 vs 15.4, p=0.39) and total time under virtual ward review (mean days 11.7 v 9.01, p=0.13). A similar proportion of people with HIV required review in the emergency department (36.2% vs 25.6%, RRR=1.45, 95%CI=0.86 to 2.33). A higher proportion of PLWH were admitted to hospital, but this did not reach statistical significance (19.0% vs. 9.30%, RRR=2.04, 95%CI=0.86 to 4.76). Of the small sample of PLWH with uncontrollable viral loads (RNA > =200), 2/5 patients (40%) were hospitalised. There were no recorded deaths.

**Conclusion:** In this cohort of mpox cases there was a high prevalence of well-controlled HIV co-infection, but we find no evidence that PLWH experience more severe mpox. Whilst there is a higher proportion of hospitalisations, this is not statistically significant and is likely to be impacted by additional caution shown by clinicians in making decisions around mpox care in these patients. All other outcomes analysed indicate that mpox infections are of similar severity in people with and without HIV, providing reassurance for patients and clinicians providing future care for patients with mpox and HIV co-infection.

### 741 ADVERSE DRUG REACTIONS ON TB TREATMENT: A PREDICTION MODEL INCLUDING HIV AND HBA1C

**Felipe Ridolfi,** Lauren Peetuk, Gustavo Amorim, Mariana Araújo-Pereira, Marcelo Cordeiro-Santos, Afranio Kritskii, Marina Figueiredo, Bruno B. Andrade, Valeria Rolila, Timothy R. Sterling

**Regional Prospective Observational Research in Tuberculosis (RePORT)-Brazil consortium**

**Background:** Adverse drug reactions (ADR) can influence treatment completion rates and the effectiveness of tuberculosis (TB) treatment. The rate of ADR related to anti-TB treatment (ATT) can be affected by HIV and diabetes mellitus. Given the paucity of prediction models for ATT-associated ADR, we developed a prediction model of ADR on TB treatment, incorporating important clinical variables.

**Methods:** We included culture-confirmed, drug-susceptible, pulmonary TB participants from the RePORT-Brazil cohort, who received standard ATT between 2015-2019. ADR was defined based on physician-assigned attribution of relation to ATT and described according to the affected organ system, HIV
status, severity, timing, and duration. Diabetes was categorized as HbA1c < 5.7% (no diabetes); ≥5.7% to < 6.5% (pre-diabetes); and ≥6.5% (diabetes). The predictive model of ADR risk used a bootstrapped backward selection approach. Of 13 candidate predictors (e.g., HIV status, HbA1c, ancestry markers), the variables retained in at least 70% of prediction models across 500 bootstrap replications were included in the final model. Model discrimination was evaluated by c-statistic and calibration with a calibration plot.

**Results:** Of 9,451 participants, 102 (1%) experienced ADR. Among 156 ADR occurrences, most (78%) were of moderate severity and occurred during the first two months of ATT (77%). Hepatic ADR were the most frequent (n=82, 53%). ADR occurred in 38 (21%) people living with HIV/AIDS (PLWHA) and in 64 (8%) HIV-seronegative. Overall, 35 (10%) normoglycemic participants had ADR, while 47 (13%) and 19% of participants with pre-diabetes and diabetes, respectively, experienced ADR. Variables included in the final prediction model for ADR were HIV status, HbA1c, age, ancestry markers, and concomitant medication use. Use of concomitant medication (mainly other antibiotics) and HIV status were highly predictive of ADR; they were included in 100% and 82% of all prediction models, respectively. The final prediction model demonstrated reasonable accuracy (c-statistic=0.75 [95% CI: 0.70-0.80]) (Figure 1A) and suitable calibration (Figure 1B). Bootstrap internal validation indicated that the model was robust (optimism-corrected c-statistic of 0.73 [95% CI: 0.68-0.78]).

**Conclusion:** We developed a robust, accurate, and reliable prediction model of ATT-related ADR. Knowledge based on important factors such as use of concomitant medication at the time of ATT initiation and HIV status could improve treatment tolerability.

Figure 1. Performance of model predicting for ADR in TB treatment. (A) The receiver operating characteristic curve indicates good fit, with c-statistic of 0.75 [95% CI: 0.70-0.80] indicating good discriminatory ability. (B) The calibration curve also indicated good fit with an optimism-corrected intercept and slope of -0.22 and 0.87, respectively.

### PREDICTORS OF POOR OUTCOMES IN PATIENTS WITH TUBERCULOSIS SYMPTOMS AT HIV DIAGNOSIS


1University of California San Francisco, San Francisco, CA, USA, 2Mbarara University of Science and Technology, Mbarara, Uganda, 3Boston University, Boston, MA, USA, 4Boston Medical Center, Boston, MA, USA, 5National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

**Background:** Standard of care has shifted to minimize delays in ART initiation. Data are limited on predictors of poor outcomes for patients who present with TB symptoms at HIV diagnosis, and initiate treatment with current, faster ATT-related ADR. Knowledge and intervention based on important factors such as use of concomitant medication at the time of ATT initiation and HIV status could improve treatment tolerability.

**Methods:** We recently completed a randomized trial to compare same-day treatment vs. standard care for participants with TB symptoms (cough, fever, night sweats, and/or weight loss) at HIV diagnosis. Both groups received a treatment vs. standard care for participants with TB symptoms (cough, fever, night sweats, and/or weight loss) at HIV diagnosis. Both groups received a digital chest radiograph, 2 Xpert Ultra tests, and 2 mycobacterial cultures within 12 days after enrollment. The current analysis includes predictors of retention, and retention with viral suppression, using univariable and multivariable models of treatment initiation.

**Results:** Of 350 participants, 102 (1%) experienced ADR. Among 155 ADR occurrences, most (78%) were of moderate severity and occurred during the first two months of ATT (77%). Hepatic ADR were the most frequent (n=82, 53%). ADR occurred in 38 (21%) people living with HIV/AIDS (PLWHA) and in 64 (8%) HIV-seronegative. Overall, 35 (10%) normoglycemic participants had ADR, while 47 (13%) and 19% of participants with pre-diabetes and diabetes, respectively, experienced ADR. Variables included in the final prediction model for ADR were HIV status, HbA1c, age, ancestry markers, and concomitant medication use. Use of concomitant medication (mainly other antibiotics) and HIV status were highly predictive of ADR; they were included in 100% and 82% of all prediction models, respectively. The final prediction model demonstrated reasonable accuracy (c-statistic=0.75 [95% CI: 0.70-0.80]) (Figure 1A) and suitable calibration (Figure 1B). Bootstrap internal validation indicated that the model was robust (optimism-corrected c-statistic of 0.73 [95% CI: 0.68-0.78]).

**Conclusion:** We developed a robust, accurate, and reliable prediction model of ATT-related ADR. Knowledge and intervention based on important factors such as use of concomitant medication at the time of ATT initiation and HIV status could improve treatment tolerability.
Conclusion: Grade 3 or higher IPT toxicities among PWH with latent TB reporting recent alcohol use were infrequent. Biomarker confirmed alcohol use was not associated with having a Grade 3 or higher toxicity. Grade 2 toxicities were more common and high/very high-risk alcohol use was associated with their occurrence. Alcohol use does not appear to pose an increased risk for serious IPT toxicity among those without significant liver enzyme elevations at baseline (<2x ULN) in PW on ART and should therefore not be deferred. Adjusted odds ratios for the associations of biomarker-confirmed alcohol use with Grade 3 and higher toxicity and Grade 2 toxicity.

<table>
<thead>
<tr>
<th>biomarker-confirmed alcohol use</th>
<th>N (%)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>Grade 3 toxicity</th>
<th>adjusted odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>96 (29.3)</td>
<td>1.00</td>
<td>0.37</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>60 (47.9)</td>
<td>1.84 (1.00-3.39)</td>
<td>1.11 (0.44-2.76)</td>
<td>1.00 (0.25-3.93)</td>
</tr>
</tbody>
</table>

**Figure 1:** Kaplan-Meier Curves for the probability of any adverse event after IPT administration

**Table 1:** RISK FACTORS FOR ADRS RELATED TO IPT AMONG PLHIV ON Dolutegravir-Based ART in Uganda

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Adjusted Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 50-59</td>
<td>1.23 (1.00-1.50)</td>
</tr>
<tr>
<td>Age &gt;60</td>
<td>1.65 (1.20-2.26)</td>
</tr>
<tr>
<td>History of Hypertension</td>
<td>1.30 (1.07-1.58)</td>
</tr>
<tr>
<td>History of Diabetes</td>
<td>1.80 (1.36-2.37)</td>
</tr>
</tbody>
</table>

745 TREATMENT OUTCOMES OF PERSONS WITH DSTB ON A MEDICATION MONITOR AND ADHERENCE SUPPORT

Salome Charalambous1, Noriah Maraba2, Lauren Jennings1, Kavindranth Veleni1, Pieter Hlupeni1, Israel Rabothata1, Rachel Mukora1, Stembridge Mpemba2, Lilile McNunu1, Dolphine Cogill, Pien Naidoo1, Catherine Orrell2, Katherine Fielding3

1The Aurum Institute, Johannesburg, South Africa, 2 Desmond Tutu Health Foundation, Cape Town, South Africa, 3 Interactive Research and Development, Johannesburg, South Africa, 4 Desmond Tutu TB Centre, Cape Town, South Africa, 5 Centre for the AIDS Programme of Research in South Africa, Johannesburg, South Africa, 6 London School of Hygiene & Tropical Medicine, London, United Kingdom

**Background:** Digital adherence technologies have gained traction recently with medication monitors showing improved adherence. Data on the impact on long-term clinical outcomes is lacking. We conducted a cluster-randomized trial to measure outcomes amongst persons with drug-susceptible TB (DS-TB) supported by medication monitors and differentiated care (intervention) versus standard care (SoC). Participants had improved adherence in the intervention versus SoC arms. Here we compare 18-month clinical outcomes by study arm.

**Methods:** DS-TB (≥2 years) persons were enrolled from 18 primary health clinics in three provinces (Gauteng, KwaZulu Natal, Western Cape) in South Africa. Intervention arm participants had visual/audio reminders for medication intake with monitoring and support depending on the number of missed doses/week. SoC arm received monitors in silent mode to document adherence. Participants were follow-up with sputum (culture) at treatment end and 18 months. Unfavorable outcome was defined as: on treatment - treatment failure, lost to follow-up, death, culture-positive at 6 months or MDR diagnosis; or recurrence to 18 months.

**Results:** We enrolled 2727 participants, reporting on 2657 participants: 38% female, median age 36 years, and 53% HIV-positive. Of 2070 participants (587 had outcome undefined), 20.9% (432/2070) had an unfavorable outcome. By arm, unfavorable outcomes were similar (geometric means: 22.3% in SoC versus 17.1% in intervention clusters). The risk ratio was 0.78 (0.53-1.16), adjusting for age, sex, TB diagnosis, ethnic group, education, marital status, HIV/ART status, and province. The effect of the intervention appeared to be greater among females and those in Gauteng and Western Cape as shown in the figure attached.

**Conclusion:** Although adherence was improved, there does not seem to be a difference in unfavorable outcomes in persons with DS-TB in the intervention versus SoC arms. Although these interventions are less likely to show an impact on clinical outcomes in routine settings, the effect on adherence is important and warrants continued use and evaluation of these technologies. Adaptation of these technologies to cater for those on both TB and HIV treatment is required.
HIGH MORTALITY IN HOSPITALISED VIRALLY SUPPRESSED ADULTS WITH HIV IN SOUTH AFRICA
Firdaus Nabeeame1, Kennedy Otombres1, Khudzadhe Hlongwane1, Nadia Sabet2, Pattammakul Ibrahim3, Alex Van Bylenstein1, Phetho Mangena4, Ebrahim Vairava5, Neil Martinson1

South Africa, 3University of the Witwatersrand, Johannesburg, South Africa, 4University of Limpopo, Polokwane, South Africa, 5Klerksdorp Tshepong Hospital Complex, Klerksdorp, South Africa

Background: Mortality in PWH has been markedly improved by antiretroviral therapy (ART) but there are few reports describing this in the ~5 million virally suppressed (VS) PWH in South Africa (SA). We describe cause of death (CoD) in adults admitted to hospital with suspected pneumonia in SA.

Methods: We enrolled patients from June 2019-October 2021 at four hospitals and then followed them up for ≥1 year. Eligibility included: Age >18 years; ≥2 signs/symptoms of pneumonia, <48 hrs since admission. Medical records were reviewed. All had HIV status ascertained and sputum sent for Mycobacterium tuberculosis (TB) and urine lipoarabinomannan were assessed. For those who died, CoD were abstracted from medical charts and interview of family. We categorised deaths as early: while admitted or to <30 days after discharge; or late: ≥30 days after discharge.

Results: Of 1999 adults, 54% were PWH; 61.2% reported receiving ART of whom 43.1% were VS; 55.5% were women. Overall median age of VS was 48 years (IQR: 40-55) at entry; 34.3% had comorbidities: hypertension (70.1%), obesity 41.3%, diabetes 28.9%). Only 11.3% were diagnosed with HIV in the past year, 35.9%, had prior TB. Median CD4 count of VS patients was 289 cells/mm³ (IQR: 133-490) and Hb, 12.5g/dL (IQR: 10.5-14.0); 53.0% had CRP >100mg/L and 69.6% had oxygen saturation <93% on room air; 14.8% had ≥1 assay positive for TB; and 42.9% were SARS-CoV-2 positive. Overall 25.4% VS PWH died compared to 31.2% and 22.9% of unsuppressed and HIV, respectively; median ages at death were 49 (IQR:43-59), 38 (IQR:32-47) and 62 (IQR:53-69) years respectively. Overall median times to early and late death was 8 (IQR:4-16) and 104 (IQR:75-254) days, respectively. The leading CoD in VS PWH were: COVID-19 (22.9%), chronic lung disease (CLD) (17.1%), malignancy (12.9%), sepsis (12.9%) and TB (8.7%); in HIV unsuppressed, CoD were: advanced HIV and opportunistic infections (TB,PJP) (55.5%), sepsis (9.6%), COVID-19 (8.6%); and in HIV: COVID-19 (43.0%), cardiovascular disease (9.0%), TB (9.0%), malignancy (8.5%).

Conclusion: Mortality in VS PWH admitted with suspected pneumonia was higher than in HIV and occurred 12 years earlier. The challenge for clinicians is to screen for diseases that disproportionately affect VS PWH and to try to prevent recurrent lung infections thereby increasing their comorbidity-free years and reduce mortality gaps.
RISK FACTORS ASSOCIATED WITH NEURO-COGNITIVE IMPAIRMENT IN CRYPTOCOCCAL MENINGITIS

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Background: The magnitude and causes of sustained neurocognitive impairment in cryptococcal meningitis are not well described. We sought to understand the clinical risk factors associated with sustained neurocognitive impairment among cryptococcal meningitis survivors.

Methods: At week 12 (+6) from diagnosis, HIV+ participants with first episode meningitis underwent neuropsychological testing consisting of a battery of tests evaluating eight neurophysiological domains. A composite quantitative neurocognitive performance z-score (QNFPZ) was calculated as the mean of eight individual z-scores. Impaired neurocognitive function is defined as a QNFPZ 8 score < -1. We compared demographic variables and clinical characteristics by QNFPZ 8 group (QNFPZ 8 < -1 vs. > = -1).

Results: QNFPZ scores of 210 participants with cryptococcal meningitis were analyzed. Overall, 72% (152/210) demonstrated sustained neurocognitive impairment at 12 weeks. There were no differences in antiretroviral therapy (ART) use (p = 0.80) or CD4 count (p = 0.36) at baseline, between the impaired and non-impaired. Impaired subjects with a QNFPZ 8 < -1 were more likely to present with a baseline Glasgow Coma Score (GCS) < 15 (p = 0.01), seizures (p = 0.04), and lower serum sodium (p = 0.03) than those non-impaired. We observed no significant difference between CSF opening pressures (p = 0.84) or quantitative CSF culture burden (p = 0.13) between impaired and non-impaired groups. We however, found that persons presenting with baseline sterile CSF cultures were more likely to be non-impaired at week 12 (p = 0.04). While we observed no differences between baseline CSF WBCs (p = 0.09) between groups, no non-impaired subjects had higher median CSF WBC on day 7 (median 25, IQR < 5 to 100 cells/mL) as compared to the impaired group (median < 5, IQR < 5 – 8 cells/mL; p = 0.03).

Conclusion: Neurocognitive impairment at week 12 is common among cryptococcal meningitis survivors. Baseline GCS < 15, seizures, and low serum sodium are risk factors for sustained neurocognitive impairment. Baseline sterile CSF cryptococcal cultures and early rise in CSF WBC is associated with better neurocognitive performance at week 12. Studies looking into the impact and degree of immune recovery during cryptococcal meningitis induction therapy to improve neurocognitive outcomes are warranted.

Table 1: Demographic characteristics by QNFPZ 8 score

| H | QNFPZ > -1 (Improved) | QNFPZ > -1 (Non-improved) | p-value
|---|-----------------------|--------------------------|--------|
| Currently on ART | 116 (69.6%) | 58 (30.4%) | 0.00
| CD4 > 200 cells/µL | 127 (71.6%) | 58 (38.4%) | 0.30
| Glasgow Coma Score < 15 | 120 (66.8%) | 58 (33.2%) | < 0.01
| Seizures | 122 (74.8%) | 58 (25.2%) | 0.04
| Serum Sodium, mmol/L | 122 (60.3%) | 58 (39.7%) | 0.03
| CSF Opening pressure, mmHg | 122 (60.3%) | 58 (39.7%) | 0.03
| CSF Cryptococcosis(CPU/mL) | 115 (64.1%) | 58 (35.9%) | 0.15
| Day 9 – Sterile CSF Culture | 151 (88.0%) | 58 (12.0%) | 0.04
| Day 9 – CSF WBC count | 149 (93.5%) | 57 (6.5%) | 0.03
| Day 7 – CSF WBC count | 149 (93.5%) | 57 (6.5%) | 0.03
| Day 14 – CSF WBC count | 149 (93.5%) | 57 (6.5%) | 0.03

1 Adjusted, p-value by Bonferroni, to control false discovery rate.

750 HIGH-DOSE ISONIAZID EARLY BACTERIAL ACTIVITY AGAINST DRUG-RESISTANT TUBERCULOSIS

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1: ACTG A5312 study team
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Background: High-dose isoniazid (INH) has been reported to be beneficial in treating multidrug-resistant tuberculosis (MDR-TB) and is part of the World Health Organization recommended shorter MDR-TB regimen. However, the optimal dose and its efficacy against katGmutated Mycobacterium tuberculosis (M. tuberculosis) are not established.

Methods: AIDS Clinical Trials Group (ACTG) A5312 is a Phase 2A randomized, open-label trial. Group 3 participants had MDR-TB with INH resistance mediated by kat6 mutation and were randomized to receive INH monotherapy doses of 15 or 20mg/kg daily for 7 days. Sputum was collected daily and processed in Mycobacteria Growth Indicator Tube to determine time to positivity (TTP). Intensive PK sampling was performed on day 6. We report here preliminary
results. INH early bactericidal activity (EBA) was estimated using daily average change in TTP between day 0 and 7 (EBATTP0−7). Linear mixed-effects modelling was used to evaluate the entire TTP profile, exploring the effect of INH concentration under concentration curve (AUC0−24) covariation with longitudinal changes in TTP.

**Results:** 21 participants were enrolled (11 in Haiti, 10 in South Africa). The majority (71%) were men, median age was 39 years, 19% were HIV-positive, and 62% had cavitary lung disease. INH AUC0-24 median (IQR) was 67 (49, 90) mg.h/L and heavily overlapped between the two dose levels. Median (IQR) EBATTP0−7 in 15 and 20mg/kg dose groups was 1.79 (−1.66, 7.31) and 2.38 (−1.29, 5.52) hours/day, respectively. Linear mixed-effects model estimated a baseline TTP of 143 hours, slope of 3.75 hours/day (corresponding to an individual with AUC0−24 of 100ng.m.L−1), and a positive relationship between higher log AUC0−24 values and increase in TTP (P=0.008). The observed INH EBA was significantly lower than our previously reported EBA of 10 hours/day in drug-sensitive TB on 5mg/kg INH. No study drug-related adverse events were observed.

**Conclusion:** Our results suggest limited to no INH EBA over 7 days against M. tb strains with katG mutations among patients with MDR-TB. The drug effect was apparent in participants with high INH AUC, which did not correlate well with dose and was highly variable, likely due to NAT2 genotype. INH is primarily activated by katG; the EBA of INH supports the hypothesis that small quantities of INH are activated either by incomplete inactivation of katG or alternative pathways. Therefore, INH may not be useful for katG-mutated M.tb as even 20mg/kg did not reach measurable EBA except in select patients.

751 **TWICE DAILY DOSING OF ATAZANAVIR SAFELY OVERCOMES THE LIMITATION WITH RIFAMPICIN**

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**Background:** Critical drug-drug interactions (DDI) exist between rifampicin and boosted protease inhibitors. Hepatotoxicity has occurred frequently in DDI studies of adjusted doses of protease inhibitors with rifampicin. We aimed to determine if dose escalation of atazanavir/ritonavir could safely overcome the DDI with rifampicin used at standard and double doses.

**Methods:** DERIVE (NCT04121195, EDCTP) was a dose-escalation trial in people living with HIV without tuberculosis on atazanavir/ritonavir-based ART in Uganda. Four intensive pharmacokinetic (PK) visits were performed at steady-state: PK1 300/100 mg OD (baseline); PK2 300/100 mg OD with rifampicin 600 mg; PK3 300/100 mg BID with rifampicin 600 mg OD; PK4 300/100 mg BID with rifampicin 1200 mg OD. Due to the potential risk of sub-therapeutic atazanavir concentrations with rifampicin, dolutegravir 50 mg BID was co-administered. Target atazanavir level against HIV is a minimum effective concentration (MEC) of 0.15 mg/L. Noncompartmental analysis was used to describe the pharmacokinetic data. Concentrations below the lower limit of quantification (LLOQ) of 0.03 mg/L were replaced by LLOQ/2.

**Results:** 26 participants were enrolled with a median (range) weight and age of 44 (28 - 61) years and 67 (50 - 75) kg, respectively, and 23 (88%) were female. Compared with PK1, atazanavir concentrations were significantly reduced at PK2: geometric mean ratio (GMR, 90%CI) of Cmin and AUC0−24 were 0.04 (0.03 - 0.05) and 0.15 (0.12 - 0.18), respectively. The escalation to BID dosing (PK3) when compared to PK1, had a GMR of 0.83 (0.68 - 1.02) and 1.08 (0.97 - 1.21), respectively. The comparable exposures were maintained with double doses of rifampicin, GMR of Cmin and AUC0−24 were 0.81 (0.67 - 1.00) and 1.01 (0.93 - 1.09) compared to PK1, respectively. The percentage of participants with concentrations below the MEC target was 4%, 100%, 23%, and 19% during PK1, PK2, PK3, and PK4 visits, respectively. No participant developed significant elevation of liver enzymes, reported an AE, or experienced rebinding viremia.

**Conclusion:** These results indicate that increasing the dose of atazanavir/ritonavir to twice daily was well-tolerated and achieved acceptable atazanavir plasma concentrations.

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752 **LINEZOLID REACHES TARGET SITE IN PATIENTS WITH TB MENINGITIS AND HIV CO-INFECTION**

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**Background:** Tuberculous meningitis (TBM) is a devastating form of TB. Several anti-TBM medications poorly penetrate the cerebrospinal fluid (CSF). Linezolid has good CSF penetration, but data for treating TBM with linezolid are limited. We assessed the pharmacokinetics (PK) and pharmacodynamics (PD) of linezolid in CSF and plasma from the ALTER trial, an ongoing Phase II, open-label, randomized clinical trial (NCT04021123) of adjunctive linezolid with high (35 mg/kg) or standard (10 mg/kg) dose rifampicin in patients with definite or suspected TBM.

**Methods:** Participants (≥18 years) were recruited from Masaka, Uganda, and were randomized 1:1 to high versus standard dose rifampicin, and then 1:1 to linezolid 1200 mg versus no linezolid for the first 4 weeks of treatment, all on a background regimen of isoniazid, pyrazinamide, and ethambutol. CSF and plasma were sampled on days 2, 14, and/or 28. We quantified the linezolid population PK in CSF and plasma, testing demographics and rifampicin dose as covariates. Clinical trial simulations (n=50, 80 patients per trial) were performed to assess the proportion of participants reaching the trial targets of AUC0−24,ss (160 µg·h/mL) and time above MIC (85% of 24h interval), and AUC0−24/MIC (100).

**Results:** Of the first 15 enrolled participants (33% women, median age 37, median weight 50 kg, 100% with HIV, median linezolid CSF and plasma peak concentrations were 3.20 and 11.0 mg/L, respectively. The PK was best described by a one compartment (distribution volume 46.1 L) model with first-order absorption (6.86 h−1), and allometrically scaled clearance (7.95 L/h) with inter-individual variability. Women had 36% lower clearance than men. Plasma-to-CSF ratio was fast, with a partition coefficient of 55%. A trend towards an effect of high dose rifampicin on linezolid PK in both CSF and plasma was observed (Figure). Based on clinical trial simulations, efficacy targets for linezolid (1200 mg QD/600 mg BID) were achieved in 30%/28% for AUC0−24,ss, 63%/87% for time above MIC, and 65%/55% for AUC0−24,MIC.

**Conclusion:** Linezolid reached the target site in patients with TB and HIV co-infection as quantified by our model-based approach of the preliminary trial data, and clinical trial simulations indicated that trial targets were achieved in a moderate to major proportion of simulated patients. Upon trial completion, this workflow will be used to confirm these results, including the effect of rifampicin dosing on linezolid PK, and support therapeutic recommendations.
Linezolid (LZD) concentrations in plasma and cerebrospinal fluid (CSF) in patients with tuberculous meningitis and HIV when given with standard (10 mg/kg) or high (35 mg/kg) dose rifampin (RIF).

753  PREDICTIVE VALIDITY OF POPULATION MODELS FOR LINEZOLID EXPOSURE AND TOXICITY IN ZeNix
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Background: Previously, using data from Nix-TB (NCT02333799, N=104), we published models relating linezolid exposure to peripheral neuropathy (PN) and hemoglobin (Hb), and we suggested how those models might guide patient management to reduce linezolid-related toxicity. Effective application of model-based insights to patient care requires model validation on independent data. Here we assess the predictive validity of the models and update them using the new and more diverse data from ZeNix (NCT03086486, N=179).

Methods: Quantifications of individual prediction errors and comparisons of observed versus simulated distributions of outcomes were used to assess the models from Nix-TB on the data from ZeNix. Models were then refitted to the combined data from ZeNix and Nix-TB and reassessed for consistency with the totality of the data. Model simulations were then compared to rates of investigator-reported adverse events in each trial.

Results: Linezolid PK in ZeNix was well predicted by the two-compartment model with nonlinear elimination developed with data from Nix-TB. The Nix-TB PK/PD model for PN predicted incidence and severity in ZeNix well for patients from South Africa on 1200 mg linezolid for 6 months, but over-predicted PN in Eastern Europe (EE) and at less intensive linezolid dosing. Refitting found both a lower overall potency of the linezolid effect on PN and a lower base probability and maximum effect for EE, and these changes improved the model fit for the combined data. The original Nix-TB model did predict the duration of PN episodes well in ZeNix. The Nix-TB PK/PD model for Hb predicted temporal distributions of Hb well in all four linezolid groups of ZeNix. The Nix-TB model’s finding that Hb change after 4 weeks of treatment was superior to linezolid trough concentrations for predicting anemia was not recapitulated in terms of the ROC AUC but did manifest in the precision-recall (PR) AUC, which emphasizes prediction of positive cases: PR AUC 0.37 (95% CI: 0.13-0.63) for percent Hb change at week 4 vs 0.15 (0.05-0.25) for observed linezolid troughs. Simulations using the refitted models for PN and Hb showed that rates of investigator-reported PN and anemia observed in the Nix-TB and ZeNix trials were well predicted (Figure).

Conclusion: Linezolid toxicity in TB is generally related to extent and duration of linezolid exposure. This work validates and refines existing models to build confidence in their application for regimen selection and patient management. Peripheral neuropathy and hemoglobin models predict rates of investigator-reported adverse events. A. Model predicted peripheral neuropathy adverse events. Bar plots show the median simulated proportion of patients with at least a minimal score for each regimen tested in Nix-TB and ZeNix and stratified by region. B. Model predicted anemia adverse events. Bar plots show the median simulated proportion of patients with at least Grade 1 anemia for each regimen tested in Nix-TB and ZeNix. Error bar shows the 95% confidence interval of the predicted median. Points are the rates of investigator-reported adverse events in the trials. Model predicted median and 95% confidence intervals were summarized based on 500 model simulations with 100 bootstrapped participants in each trial.
755 EFFECTIVENESS OF DULUTEGRAVIR IN PEOPLE ON RIFAMPIN-BASED TUBERCULOSIS TREATMENT

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Background: Tenofovir-lamivudine-dolutegravir (TLD) is the WHO-preferred first-line regimen for people with HIV, but drug-drug interactions between dolutegravir (DTG) and rifampin (RIF) require an additional 50mg DTG (TLD+50) in people receiving tuberculosis (TB)/HIV co-treatment. RIF is a key drug in TB treatment, but is a potent inducer of metabolizing enzymes and efflux transporters, which can markedly lower drug concentrations. There are limited data on the effectiveness of TLD+50 in people with TB/HIV from program settings.

Methods: We conducted a prospective, observational study at 12 sites in 6 countries (Haiti, Kenya, Malawi, South Africa, Uganda, Zimbabwe). Participants received concomitant TLD+50 and RIF-based TB treatment provided as standard of care by HIV and TB treatment programs. Primary outcome was HIV-1 RNA <1000 copies/mL (cpm) at end of TB treatment. New DTG resistance mutations were detected among 4 participants with HIV-1 RNA >1000 cpm.

Results: From November 2016 through February 2018, 199 participants with MDR-TB and HIV were enrolled and followed through treatment completion (median 17.2 months: IQR 12.2–19.6). 12 focus groups were conducted. While the majority (83.2%, 166/199) maintained high adherence, a severely adherence challenged subpopulation (16.8%, 33/199) had a precipitous decline in mean BDQ adherence from 91.9% to 44.7% and mean ART adherence from 84.5% to 21.6% over six months (F1, Panel A, B). Qualitative analysis identified discrete treatment stages associated with specific barriers (F1, Panel C) which, when aligned with quantitative data, suggests that declining medication adherence may relate to psychosocial, behavioral, and structural barriers.

Conclusion: Based on these data, MDR-TB HIV DSD frameworks should 1) intensify support for severely adherence challenged subpopulations while adherent patients may require less intensive support, 2) address decreased adherence over time and 3) account for psychosocial, behavioral, and structural challenges linked to discrete treatment stages. DSD models that offer evaluation and intervention at key stages, tailored to needs of both vulnerable and adherent populations, have the potential to improve adherence and outcomes in MDR-TB HIV treatment.
ACCELERATING TUBERCULOSIS PREVENTIVE TREATMENT AMONG TANZANIANS LIVING WITH HIV

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Background: People living with HIV (PLHIV) bear 20 times higher risk of acquiring tuberculosis (TB) compared to people without HIV. The World Health Organization recommends TB preventive treatment (TPT) for PLHIV to reduce this risk. However, according to the 2020 Global TB Report, only half of PLHIV were started on TPT globally in 2019, with the lowest coverage observed in low-income countries including Tanzania, where TPT provision is part of the standard of care for eligible PLHIV in Tanzania. We describe programmatic efforts to scale up TPT in 11 regions accounting for half of the 1.5 million PLHIV on ART in Tanzania.

Methods: Starting in 2018, PEPFAR, through the U.S. Centers for Disease Control and Prevention (CDC) supported the Government of Tanzania to accelerate TPT provision by: (1) training and mentoring healthcare workers, (2) integrating isoniazid into supply chain plans at the regional level, and (3) convening quarterly meetings at national and regional levels for program and supply chain monitoring and coordination. Additionally, CDC launched focused regional support interventions, with TPT among its priorities, aiming to accelerate TPT provision by: (1) training and mentoring healthcare workers, (2) integrating isoniazid into supply chain plans at the regional level, and (3) convening quarterly meetings at national and regional levels for program and supply chain monitoring and coordination. Additionally, CDC launched focused regional support interventions, with TPT among its priorities, aiming to facilitate real-time data-driven site monitoring, increased accountability, and on-the-ground coordination with local health authorities and implementing partners. We analyzed routine programmatic data reported in PEPFAR’s reporting system for fiscal years (FY) FY 2018 through FY 2021.

Results: The number of PLHIV of all ages who initiated TPT increased from 67,510 in FY 2018 to 268,909 in FY 2019. Despite coinciding with the COVID-19 pandemic, the initiation numbers in FY 2020 were sustained at 264,465 and dropped by about one-third in FY 2021 (182,823) compared to the previous year. TPT completion rates among those initiated also showed a positive trend; 38% in FY 2018, 85% in FY 2019, 90% in FY 2020, and 91% in FY 2021.

Conclusion: Our findings demonstrate substantial acceleration of TPT initiation and a significant increase in TPT completion rates over the four-year period in 11 regions in Tanzania. We identify the policy of once-in-a-lifetime TPT for PLHIV means fewer people are eligible for TPT over time, which might account for lower numbers of PLHIV initiated on TPT in FY 2021. Completion remained high among those who initiated TPT. The strategic shift focusing on capacity building, supply chain strengthening, and site-level monitoring may have contributed to the improvements in TPT initiation and completion.

MONOCYTE-TO-LYMPHOCYTE RATIO AND HEMOGLOBIN LEVEL TO PREDICT TB AFTER ART INITIATION

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Background: Monocyte-to-lymphocyte ratio (MLR) and hemoglobin level are commonly used parameters to predict tuberculosis (TB) disease in HIV-infected patients. These findings suggest that these clinical variables could be used to predict TB incidence among people living with HIV (PLHIV). The World Health Organization recommends TB preventive treatment (TPT) for PLHIV to reduce this risk. However, according to the 2020 Global TB Report, only half of PLHIV were started on TPT globally in 2019, with the lowest coverage observed in low-income countries including Tanzania, where TPT provision is part of the standard of care for eligible PLHIV in Tanzania. We describe programmatic efforts to scale up TPT in 11 regions accounting for half of the 1.5 million PLHIV on ART in Tanzania.

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Conclusion: Our findings demonstrate substantial acceleration of TPT initiation and a significant increase in TPT completion rates over the four-year period in 11 regions in Tanzania. We identify the policy of once-in-a-lifetime TPT for PLHIV means fewer people are eligible for TPT over time, which might account for lower numbers of PLHIV initiated on TPT in FY 2021. Completion remained high among those who initiated TPT. The strategic shift focusing on capacity building, supply chain strengthening, and site-level monitoring may have contributed to the improvements in TPT initiation and completion.

Patients and Methods: We conducted a retrospective (January 2018 – February 2022) cohort analysis of the Ukraine national HIV and TB electronic case-based surveillance database, where all PLHIV are enrolled. TPT was categorized as complete if documentation confirmed adherence to >= 146 days of isoniazid, partial if 28–146 days, and none if < 28 days. TB incidence rates (cases per 100 person-years) and incidence rate ratios (IRR) were calculated for each of the TPT completion categories using a Poisson model, adjusting for demographic and clinical variables.

Results: Of the 166,365 PLHIV, 123,884 (74.5%) included complete data on TPT duration and TB diagnosis. Overall, 24.7% completed TPT, 9.6% had partial completion, and 65.7% did not receive TPT. Adjusted TB incidence rate was 1.9, 2.5, and 5.0 among those that completed, partially completed (IRR 1.45), and did not receive TPT (IRR 2.58), respectively. Among PLHIV with newly diagnosed TB, drug resistance occurred in 21.9%, 20.4%, and 21.6% among those that completed, partially completed, and did not receive TPT, respectively.

Conclusion: In Ukraine, TB incidence showed a dose response relationship to TPT duration and was lowest in PLHIV who completed TPT. Multidrug resistance was commonly observed in this setting and comparable across TPT groups. These findings suggest TPT may benefit PLHIV in high-burden MDR-TB settings.
Methods: We previously identified an MLR threshold ≥ 0.23 optimally predicted incident TB after ART initiation. In this study, we used ACTG A5175 trial data as a validation cohort. We assessed the utility of baseline MLR and anemia severity, alone and in combination, for predicting incident TB in PWI in the first year after ART initiation. Participants starting ART were included in this analysis if they had no active TB at study entry or the 12 months before enrollment. Cox regression was used to assess associations of MLR and anemia severity with incident TB. Harrell’s C index was used to describe single model discrimination and model prediction was compared using log-likelihood based methods.

Results: Total of 1,455 participants were included. Median (IQR) age was 34(29,41) years; baseline CD4 was 174(92,234) cells/μL; 1,246(86%) participants were from high TB burden countries and 48% were women. Fifty-four participants were diagnosed with TB within 1 year of ART initiation. Median time from ART start to TB diagnosis was 4.1 (IQR 1.3, 8.4) months. The hazard ratio (HR) for incident TB was 1.36 (95% CI; 1.05-1.78), and increasing degrees of anemia severity (aHR 1.34(95%CI; 1.10-1.67), p < 0.01) and severe anemia (aHR 2.31(95%CI; 1.77-3.02), p < 0.001) for severe anemia. After combining parameters, there were small increases in adjusted HR (aHR) for MLR ≥ 0.23 to 1.86(95%CI; 1.05-3.33), and increasing degrees of anemia severity (aHR 2.56(95%CI; 1.39-4.68), p < 0.001). After adjusting for both factors, the model AIC decreased from 792.37 to 785.56 after addition of MLR (P = 0.03).

Conclusion: Addition of MLR to anemia severity improved prediction of incident TB. Routinely measured MLR and hemoglobin levels should be assessed at ART initiation to help identify those who would benefit from TB preventive interventions.

The Cox proportional hazard model and C index of MLR and anemia severity for incident tuberculosis among participants.

Table: Cox proportional hazard model and C index of MLR and anemia severity for incident tuberculosis among participants

<table>
<thead>
<tr>
<th>Model</th>
<th>Hazard Ratio (95% CI)</th>
<th>P value</th>
<th>Hazard Ratio (95% CI)</th>
<th>C index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.36 (1.05-1.78)</td>
<td>0.010</td>
<td>0.57</td>
<td>0.55 (0.50-0.60)</td>
</tr>
<tr>
<td>MLR</td>
<td>1.34 (1.10-1.67)</td>
<td>&lt;0.001</td>
<td>0.81</td>
<td>0.81 (0.76-0.86)</td>
</tr>
<tr>
<td>Anemia</td>
<td>2.31 (1.77-3.02)</td>
<td>&lt;0.001</td>
<td>0.88</td>
<td>0.82 (0.76-0.89)</td>
</tr>
<tr>
<td>MLR + Anemia</td>
<td>2.56 (1.39-4.68)</td>
<td>&lt;0.001</td>
<td>0.89</td>
<td>0.89 (0.84-0.94)</td>
</tr>
</tbody>
</table>

760 IMMUNEOMIC PROFILES TO DISTINGUISH TUBERCULOSIS AMONG ADVANCED HIV ADULTS IN INDIA

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Background: Diagnosis of tuberculosis (TB) in people with HIV (PWH) with significant immunosuppression remains a challenge. Integration of flow cytometry, cytokine, and gene expression data is a promising approach for developing diagnostic TB biomarkers but such “multi-omic” analysis remains poorly explored.

Methods: We enrolled 30 adults with advanced HIV(CD4 < 100 cells/μL) in India; 16 with microbiologically confirmed active pulmonary TB and 14 without. Transcriptomics (RNA-seq), flow cytometry to track T-cell (CD4+/CD8+) activation markers (CD38/HLADR-), and Luminex-based measurement of plasma cytokines/chemokines performed at baseline and week 24 of anti-TB therapy. Comparisons between HIV and TBHIV groups performed using the Mann-Whitney U test. Feature selection analysis with a random forest algorithm was employed to determine the most predictive variables for classification of HIV and TBHIV. Accuracy was depicted using c-statistics. Associations between measurements of biomarkers in different assays were assessed using Spearman correlation networks defined with False Discovery Rate < 0.05.

Results: Comparison between HIV and TBHIV did not show significant differences in the expression of CD38 + HLA-DR+ on CD4+/CD8+ T cells. Levels of CRP, CXCL10, IL6, CD14, and TNF were higher in TBHIV at baseline and week 24. Baseline levels of CXCL10 and TNF accurately identified TB cases with an AUC of 0.89. Age, BMI, and Viral load values did not directly influence cytokine levels. While examining relationships between protein, cellular markers, and overall profile of gene expression, we noted only TBHIV group exhibited statistically significant correlations. Pathway enrichment analysis uncovered a variety of processes associated with innate immunity, such as neutrophil degranulation and toll-like receptor cascades, hallmarking the transcriptional profile of TBHIV but not in HIV without TB(Fig1).

Conclusion: Circulating levels of TNF and CXCL10 are uniquely distinct among PWH with advanced disease with or without TB coinfection both at baseline and week 24 of anti-TB therapy. Integrative multi-omic analysis suggests a distinctive inflammatory profile is linked to the activation of key processes related to innate immunity. Major contributions of the findings presented are twofold as they (i) reveal parsimonious biomarker signatures highly accurate in identifying TB cases among PWH with advanced disease (ii) underscore biologic pathways that may further explore as therapeutic targets to reduce disease severity.

761 PERFORMANCE OF URINE XPERT ULTRA VS ALERE LAM FOR DIAGNOSING TB IN HIV IN-PATIENTS

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1Frere Hospital, East London, South Africa, 2Groote Schuur Hospital, Cape Town, South Africa, 3University of Cape Town, Cape Town, South Africa, 4Civica Makwane Hospital, East London, South Africa, 5Background: Rapid urine-based TB diagnostics, including lateral flow urinary lipoarabinomannan (LF-LAM), reduce mortality in HIV-positive in-patients. Xpert MTB/RIF Ultra has improved sensitivity on sputum, and may be a useful urine-based diagnostic test for disseminated TB.

Methods: We conducted a cross-sectional diagnostic accuracy study at two hospitals in East London, South Africa. From August 2018 – February 2019, consecutive HIV-positive adults admitted with ≥1 WHO TB symptom or clinical concern for TB were enrolled and underwent TB cultures of blood, sputum, and urine. Urine was obtained for bedside urine Alere LF-LAM testing and Ultra was performed on the pellet of 15ml centrifuged urine. Vital status was assessed by phone call at 12 weeks. Diagnostic classifications were: ‘definite TB’ (positive TB culture or molecular test), ‘probable TB’ (clinical-radiological features of TB and response to TB therapy), and ‘not TB’ (remained well without TB therapy). The primary outcome was sensitivity of urine Ultra for definite TB. We also calculated diagnostic yield (proportion positive tests among TB cases) and compared the diagnostic performance of urine-based tests and sputum Ultra.

Results: 238 participants were enrolled. Median age was 39 years (IQR 32–48), 124 (52%) female, median CD4 count 76 cells/μL (IQR 22-203). Definite TB was diagnosed in 62 (26%) and either definite or probable TB in 92 (39%). Diagnostic yields for definite TB were 34% (n=21) for sputum Ultra, 45% (n=28) for urine LF-LAM, 68% (n=42) for urine Ultra, and 73% (n=45) for urine LF-LAM and urine Ultra combined. The sensitivity and
specificity using definitive TB as a reference were 55% and 90% for urine LF-LAM, and 70% and 100% for urine Ultra, respectively. Positive urine Ultra results were 68.5% (n=37) rifampicin susceptible, 9.3% (n=5) resistant, and 22.2% (n=12) indifferent, with no discordances with culture or sputum Ultra rifampicin susceptibility results.

Conclusion: Combined urine testing (Ultra + LF-LAM) identified three-quarters of HIV-positive medical in-patients with definite TB. Urine Ultra had improved sensitivity and specificity compared to LF-LAM and has added benefit of providing rapid rifampicin susceptibility results.

762 CSF IMMUNE RESPONSE ASSOCIATED WITH FUNGAL BURDEN IN CRYPTOCOCCAL MENINGITIS

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University of Minnesota, Minneapolis, MN, USA, Makerere University, Kampala, Uganda, Infectious Disease Institute, Kampala, Uganda

Background: Cryptococcal meningoencephalitis causes substantial mortality in persons with advanced HIV. The cerebrospinal fluid (CSF) cellular immune response in cryptococcal meningoencephalitis remains poorly defined. We used flow cytometry to characterize the immune response in CSF and assessed correlations with baseline cryptococcal CSF fungal burden.

Methods: CSF was obtained from 20 patients with HIV-associated cryptococcal meningoencephalitis diagnosed by CSF cryptococcal antigen and quantitative CSF fungal culture. We centrifuged and fixed cells within <1 hour of collection. We stained fixed CSF cells pellets with a repertoire of antibodies to identify innate and adaptive immune cells and analyzed cells on a 13-color flow cytometer. We calculated absolute cells/mL of CSF by dividing sample cell counts by the pre-centrifuged CSF volume. We determined the correlation between the CSF cellular subsets and cryptococcal CSF fungal burden by Spearman’s rank testing.

Results: In blood, the median CD4 count was 25 cells/µL (range: 3-380 cells/µL, IQR 41.5). In CSF, the median Cryptococcus quantitative culture was 17,875 CFU/mL (range: 0-985,000 CFU/mL), and the median absolute white blood cells (CD45+) was 17,303 cells/mL with 111 CD4+ cells/mL and 2,531 CD8+ cells/mL. We found a moderate negative correlation between baseline CSF quantitative culture and absolute CSF white cells/mL (Spearman rho = −0.58, P = 0.007).

Conclusion: Our findings suggest a higher CSF fungal burden correlates with a weakened immune response at the site of infection. A subset of CSF CD45+ cells are probable Cryptococcus yeast; however, further investigation is required for the future validation of CD45 as a flow cytometry real-time marker of fungal burden instead of quantitative CSF culture that can take up to 10 days.

763 COST-EFFECTIVENESS OF CRAg SCREENING FOR PLHIV WITH ADVANCED HIV DISEASE IN MALAWI

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University of Massachusetts General Hospital, Boston, MA, USA, Centers for Disease Control and Prevention, Cape Town, South Africa, Elisabeth Glaser Pediatric AIDS Foundation, Lilongwe, Malawi, World Health Organization, Geneva, Switzerland, Elisabeth Glaser Pediatric AIDS Foundation, Washington, WA, USA, University College London, London, United Kingdom, Harvard Medical School, Boston, MA, USA

Background: Cryptococcal meningitis (CM) remains a leading cause of death among PLHIV; diagnosis and treatment of asymptomatic cryptococcal disease can prevent progression to symptomatic CM. We projected the clinical impact and cost-effectiveness of cryptococcal antigen (CRAg) screening with preemptive fluconazole therapy in PLHIV with CD4<200 µL and no meningitis symptoms initiating ART in Malawi.

Methods: We used the validated CEPAc model to examine 2 strategies at ART initiation: 1) no CRAg screening and 2) a serum CRAg screening test. The simulated population was 51% female, age >15y (mean age 37y), mean CD4 97µL, including 4% with cryptococcal infection (3%, asymptomatic CM; 69% cryptococccemia alone). Progression to symptomatic CM occurs more frequently in asymptomatic CM (80% without preemptive fluconazole (FLU; ≥50), 25% with FLU) vs cryptococccemia (7% without FLU, 0% with FLU). Serum CRAg (Se 97.6; Sp 98.1; $4.70/test) has 95% testing uptake and 80% preemptive fluconazole initiation. PLHIV who progress to symptomatic CM receive amphotericin B/fluconazole (AmB/FLU, 35% mortality; $590). Model outcomes included 1 year survival, CM deaths, life expectancy, costs, and incremental cost-effectiveness ratios (ICER, $/year of life saved (YLS)); we considered ICERS < $640 (Malawi 2021 per capita GDP) to be cost-effective. We evaluated single-dose liposomal AmB (LAmB) with flucytosine/FLU (17% mortality; $1100) as preferred CM treatment. We evaluated key input parameters in sensitivity analyses.

Results: Compared with no CRAg screening, CRAg screening would result in 23.6% reduction in CM deaths, 0.10 years of life saved (YLS), and would be cost-effective (ICER, $270/YLS). In people with asymptomatic cryptococcal disease, CRAg screening would reduce CM deaths by 39.7%, resulting in 1.80 YLS. Screening remained cost-effective with LAmB-based CM treatment. In sensitivity analysis, screening was cost-effective at 1x per capita GDP even at asymptomatic cryptococcal prevalence < 1% or when linkage to FLU preemptive therapy was >50%. Sex at birth; LAmB cost; and CRAg cost, sensitivity, and specificity did not substantially impact cost-effectiveness.

Conclusion: Serum CRAg screening at ART initiation would offer substantial clinical benefits and would be cost-effective in Malawi. Screening would likely remain cost-effective at lower prevalence and linkage, and as LAmB-based CM treatment becomes more widely available.

Table 1. Clinical benefits, costs, and cost-effectiveness of CRAg screening among people with advanced HIV Disease (AIDS) in Malawi.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Survival at 1 Year (%)</th>
<th>Reduction in CM Deaths (%)</th>
<th>Total YLS gained</th>
<th>Total YLS saved</th>
<th>Cost, $/YLS</th>
<th>Cost, $/YLS, LAmB, EYLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CRAg screening</td>
<td>89.0</td>
<td>17.99</td>
<td>11.64</td>
<td>2070</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>No CRAg screening, no ART</td>
<td>89.5</td>
<td>23.6</td>
<td>16.69</td>
<td>17.70</td>
<td>2090</td>
<td>270</td>
</tr>
<tr>
<td>Cryptococcal disease cohort</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No CRAg screening, no ART</td>
<td>82.7</td>
<td>11.34</td>
<td>7.40</td>
<td>1470</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>No CRAg screening, to ART</td>
<td>72.9</td>
<td>39.17</td>
<td>13.86</td>
<td>8.56</td>
<td>1680</td>
<td>190</td>
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<tr>
<td>Scenario analysis</td>
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<tr>
<td>No CRAg screening, to ART</td>
<td>89.5</td>
<td>23.3</td>
<td>16.09</td>
<td>17.70</td>
<td>2090</td>
<td>270</td>
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<tr>
<td>Sensitivity analyses</td>
<td></td>
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<tr>
<td>Prevalence of asymptomatic cryptococcal disease (base case is 4%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1% No CRAg screening</td>
<td>89.0</td>
<td>16.20</td>
<td>11.77</td>
<td>2060</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1% No CRAg screening, no ART</td>
<td>89.0</td>
<td>16.20</td>
<td>11.77</td>
<td>2060</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>8% No CRAg screening</td>
<td>87.9</td>
<td>17.71</td>
<td>11.46</td>
<td>2040</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>8% No CRAg screening, no ART</td>
<td>87.9</td>
<td>17.71</td>
<td>11.46</td>
<td>2040</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Linkage to preemptive fluconazole after CRAg screening (base case is 80%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50% No CRAg screening</td>
<td>89.3</td>
<td>12.7</td>
<td>10.05</td>
<td>11.67</td>
<td>2080</td>
<td>310</td>
</tr>
<tr>
<td>50% No CRAg screening, no ART</td>
<td>89.3</td>
<td>12.7</td>
<td>10.05</td>
<td>11.67</td>
<td>2080</td>
<td>310</td>
</tr>
<tr>
<td>95% No CRAg screening</td>
<td>88.9</td>
<td>17.88</td>
<td>11.64</td>
<td>2070</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>95% No CRAg screening, no ART</td>
<td>88.9</td>
<td>17.88</td>
<td>11.64</td>
<td>2070</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* We considered ICERs < $640 (Malawi 2021 per capita GDP) to be cost-effective.

** Lifetime horizon. *Annual discount rate of 2%.

Abbreviations: CRAg: cryptococcal antigen; CM: cryptococcal meningitis; LC: life year unit; undiscounted; undiscounted; disc: discounted; EYLD: years of life saved; AmB: amphotericin B; FLU: fluconazole; LAmB: liposomal amphotericin B; SCF: fluconazole.

764 MYCO/F LYTIC VS STANDARD BLOOD CULTURES IN FUNGAL DETECTION IN AIDS PATIENTS


Background: Invasive mycoses are prevalent in hospitalized patients with advanced HIV disease, but fungal blood culture is rarely performed in Southeast Asia. The standard 5-day incubation of the BD BACTEC or BACT/ALERT blood culture is infrequently performed in HIV-positive medical in-patients with definite TB. Urine Ultra had improved sensitivity and specificity compared to LF-LAM and has added benefit of providing rapid rifampicin susceptibility results.

Methods: We prospectively enrolled adult patients with CD4 ≤ 100 cells/µL or WHO stage III or IV disease, were not on ART or were on ART for ≤ 3 months or >12 months, who were hospitalized at the National Hospital for Tropical Diseases (NHTD) in Hanoi and the Hospital for Tropical Diseases in Ho Chi Minh. Myco/F Lytic and standard blood culture were performed for all study participants. Incubation was up to 5 days and 42 days for standard bottles and Myco/F Lytic bottle, respectively. For all positive bottles, microscopy with Gram and Giemsa stains, followed by standard MALDEFIT identification and fungal...
sub-culturing on SDA were performed to demonstrate dimorphism. The two culture procedures were compared for the number of positive fungal blood cultures using Student’s t-tests, and culture turnaround time was described.

**Results:** We recruited 319 eligible patients between 22 Feb 2021 and 31 May 2022. A total of 71 patients (22.3%) had a fungal pathogen detected in blood culture: 59/71 cases (83.1%) had Tm; 60/71 cases (8.5%) had Hc, and 06/71 cases (8.5%) had C. neoformans. Of the 71 fungal infections, the standard blood cultures only detected 40 (56.3%) cases while Myco/F Lytic blood culture detected 68 (95.8%) cases. The median time to detection of Tm cases and Hc cases by Myco/F Lytic blood culture was 13 days (IQR 8 – 17.5) and 31 days (IQR 23.5 – 45.5), respectively.

**Conclusion:** We report a high prevalence of talaromycosis (16.7%) in hospitalized patients with advanced HIV disease. Tm was most prevalent (83/1), followed by Hc and Cryptococcus (each 8.5%). Myco/F Lytic blood culture detected 25 additional Tm cases and all 6 Hc cases. A total of 43.7% of infections would have been missed by standard blood cultures. Our data demonstrate the need to urgently implement Myco/F Lytic blood culture system into routine diagnostic for hospitalized patients with advanced HIV disease in Southeast Asia.

Venn diagram demonstrating the detection of invasive mycoses by the standard versus Myco/F Lytic blood culture systems.

**765 MULTICENTER PROSPECTIVE VALIDATION STUDY OF A NOVEL ANTIGEN ASSAY FOR TALAROMYCOSIS**

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**Background:** Talaromycosis – caused by the dimorphic fungus *Talaromyces marneffei* (Tm) endemic in Southeast Asia - is a leading cause of death in patients with advanced HIV disease (AHD). Blood culture is the mainstay of diagnosis but is only positive during late-staged infection and can take up to 28 days, leading to delayed treatment and high mortality. We report interim results of an ongoing multi-center diagnostic validation study of a novel Tm-specific Mp1p antigen enzyme immunoassay (EIA) for diagnosis of talaromycosis.

**Methods:** We prospective recruited hospitalized adult patients with AHD (CD4 < 100 cells/µL or WHO stage III/IV disease) who were ART-naïve or on ART for ≤3 months or <12 months at the National Hospital for Tropical Diseases in Hanoi and the Hospital for Tropical Diseases in Ho Chi Minh City. Mp1p EIA was performed on serum and urine samples at enrollment for all patients. Talaromycosis is diagnosed by conventional blood culture for all patients, alongside microscopic examination and cultures of skin lesions, lymph node, bone marrow or other body fluids as clinically indicated. We followed patients monthly over 6 months for development of culture-confirmed talaromycosis.

**Results:** Of 432 patients recruited between Feb 2021 and July 2022, 426 patients were included in the analysis; 3505 women were included in the analysis; 305 women were PrEP-unexposed and 103 PrEP initiators were randomly selected and had detectable TFV-DP during pregnancy (18% of all PrEP initiators). Median maternal age at enrollment was 24 years (IQR: 21-28), median gestational age was 24 weeks (IQR: 21-28) and 27% were primigravid. Compared to PrEP-unexposed women, women with confirmed PrEP exposure during pregnancy experienced similar frequencies of stillbirth (4% vs. 3%, aPR=1.03, 95% CI 0.89-1.2, p=0.46), preterm birth (16% vs. 15%, aPR=0.95, 95% CI 0.58-1.54, p=0.78), low birthweight (6% vs. 2%, aPR=0.38, small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%, aPR=0.81, 95% CI 0.54-1.2, p=0.38), preterm birth (6% vs. 5%, aPR=0.73, p=0.78), small-for-gestational-age (13% vs. 10%,
767 HIGH ACCEPTABILITY OF STI TESTING AND EPT AMONG PREGNANT KENYAN WOMEN INITIATING PrEP

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Methods: A recent systematic review found low acceptability of STI screening in pregnancy. The objective of this study was to investigate STI testing and EPT among pregnant women initiating PrEP.

Methods: Between November 2021 and January 2023, pregnant women ≥16 years old, intending to initiate PrEP, who agreed to participate in a 2-month search for STIs were enrolled. STIs were confirmed by Xpert CT/NG testing.

Results: Of 1200 pregnant women enrolled, 1193 (99.4%) reported STI testing and 907 (75.4%) had a STI diagnosed. Among women who tested positive, 724 (80.0%) reported a STI treatment. Among women who tested negative, 221 (24.5%) reported to have been tested for STI in the past. Women who tested positive for STIs were more likely to have a STI treatment (aOR=3.00; 95% CI=2.51, 3.58, p<0.001) and had a higher likelihood of PrEP acceptance (aOR=1.66; 95% CI=1.25, 2.21, p=0.001).

Conclusion: STI testing and EPT acceptance was high among pregnant women initiating PrEP. STI testing and EPT may improve PrEP continuation.

768 INTEGRATING PrEP INTO ANTENATAL CARE FOR HIV-NEGATIVE PREGNANT WOMEN IN SOUTH AFRICA

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Methods: A stepped-wedge cluster randomized pragmatic trial to catalyze scale-up of PrEP delivery integrated in 25 public HIV clinics in Kenya. We examined the pattern and relation between PrEP continuation and fertility intentions, pregnancy and breastfeeding status during the first year of PrEP.

Results: A total of 2640 women initiated PrEP; median age was 31 years (IQR 24-36), most (80%) were in serodifferent relationships, 44% reported inconsistent condom use, and 12% reported multiple sex partners. Overall, 11% were pregnant, and 16% were breastfeeding; among non-pregnant women at baseline (n=1319), 15% were actively trying to conceive, 25% were breastfeeding status during the first year of PrEP.

Conclusion: Despite high PrEP initiation at first ANC visit, almost one-fifth did not return for the 1-month refill visit, and < 50% continued after 6 months when all were in postpartum period. A subset of women started PrEP in postpartum period, or restarted PrEP in postpartum. There is an urgent need for PrEP integration into antenatal and postpartum care including interventions to improve PrEP continuation.
future pregnancy intention, and 33% had no pregnancy intention. Among all women, PrEP continuation at 1, 3, and 6 months was 59%, 45%, and 36%, respectively, and did not differ by breastfeeding or pregnant status. At 1, 3 and 6 months, PrEP continuation rates for women who were neither pregnant nor breastfeeding were 59%, 43%, and 35%; pregnant: 65%, 49%, and 35%; breastfeeding: 59%, 42%, and 34%, respectively (p > 0.05 for all comparisons). Among non-pregnant women at baseline, continuation was higher for those actively trying to conceive versus those with future conception plans and those with no plans for conceiving: 70%, 61%, and 58% at month 1; 55%, 44%, and 44% at month 3; and 46%, 34% and 37% at month 6, respectively (p < 0.01 for all comparisons).

Conclusion: In this large real-world PrEP implementation program in Kenya, intention to conceive was associated with better continuation on PrEP and pregnancy and breastfeeding periods had similar continuation patterns on PrEP as non-pregnant periods.

### 770 PrEP LEVELS STRONGLY CORRELATED IN HAIR AND DBS DURING PREGNANCY AND POSTPARTUM

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**Background:** Lower tenofovir (TFV) & metabolite concentrations have been observed in plasma and dried blood spots (DBS) among women during pregnancy compared to non-pregnant periods. Hair TFV levels, which measure long-term adherence, may be less affected by physiologic changes during pregnancy that can influence blood-based measures. To date, PrEP levels have not been compared in hair and DBS during pregnancy and postpartum.

**Methods:** The PrEP Implementation for Mothers in Antenatal Care Study evaluated PrEP delivery strategies for pregnant women who were followed 9-months postpartum. Hair and DBS samples were collected at visits from a subset of women who reported using PrEP in the last 30 days. PrEP drug levels were measured using liquid chromatography/tandem mass spectrometry. The correlations between TFV levels in hair and tenofovir-diphosphate (TFV-DP) levels in DBS were calculated using the Spearman coefficient. Median hair TFV levels were calculated based on benchmarks for PrEP dosing in DBS among pregnant/postpartum women in the IMPAACT 2009 study and hair benchmarks in non-pregnant women.

**Results:** Overall, 34 hair-DBS paired samples were evaluated; 12 (35%) from pregnancy visits at a median of 32 weeks gestation and 22 (65%) from postpartum visits at a median of 3.5 months since birth. Median time since PrEP initiation was 18 weeks (IQR: 7-33) at sample collection. TFV levels in hair were strongly correlated with TFV-DP levels in DBS (r = 0.77, p < 0.001), with stronger correlation postpartum (r = 0.82, p < 0.001) compared to pregnancy (r = 0.57, p = 0.05). Based on DBS benchmarks, 44% of DBS samples had TFV-DP levels indicative of ≥2 doses/week; 41% of hair samples had TFV levels indicative of ≥2 doses/week based on benchmarks from non-pregnant women. Median hair TFV levels for women who took < 2 and ≥2 doses/week were 0 ng/mg (IQR: 0-0.006, n=19) and 0.035 ng/mg (0.021-0.039, n=15), respectively, based on DBS benchmarks; results were similar by pregnancy status (0.029 ng/mg pregnancy vs. 0.035 ng/mg postpartum, p = 0.37, Table 1).

**Conclusion:** Our findings suggest that hair PrEP measures are strongly correlated with DBS and may not need adjustment for PK differences over the perinatal period when used as adherence metrics. These data suggest an advantage of using hair measures for PrEP adherence during pregnancy and postpartum over blood-based measures which are more influenced by physiologic changes during the perinatal period.

### 771 LONGITUDINAL PRÉP ADHÉRENCIE AMONG KENYAN WOMEN WHO INITIATED PRÉP DURING PREGNANCY

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**Background:** PrEP is scaling up among pregnant and postpartum women in Kenya, yet few longitudinal data exist on PrEP adherence in this population. We evaluated PrEP adherence measured via tenofovir-diphosphate (TFV-DP) concentrations in dried blood spots (DBS) collected from Kenyan women who initiated PrEP during pregnancy and were followed postpartum.

**Methods:** We prospectively analyzed data from a subset of participants in the PrIMA Study (NCT03070600) who enrolled during the 2nd trimester, initiated PrEP during pregnancy, and were followed through 9-months postpartum. At follow-up visits (monthly in pregnancy; 6 weeks, 6 months, 9 months postpartum), self-reported PrEP use was assessed and DBS were collected. Among a random subset of participants, DBS quantifying TFV-DP concentrations were tested from all visits with any self-reported PrEP use in the last 30 days. TFV-DP benchmarks were defined by thresholds from directly observed pharmacokinetic studies among non-pregnant women (Luo et al. 2014 and Kiss et al. 2010) as benchmarks for pregnant women are unavailable.

**Results:** Overall, 198 participants met inclusion criteria for this analysis and were randomly selected (28% of all PrEP initiators in PrIMA); each participant contributed a median of 3 visits to the analysis (IQR: 2-4). The median gestational age at PrEP initiation was 27 weeks (IQR: 22-30); 91% of participants were married, and 19% had a partner known to be living with HIV. Among visits where participants continued with PrEP (n = 454), 94% (427/454) reported any PrEP use in the last 30 days. Among DBS from these visits (n = 427), 48% had detectable TFV-DP of which 26% had TFV-DP concentrations indicating < 2 doses/week, 64% ≥2-6 doses/week, and 8% ≥7 doses/week. Having a partner known to be living with HIV was associated with a 2-fold higher likelihood of any detectable TFV-DP compared to having partners who were HIV-negative or of unknown HIV status (risk ratio [RR] = 2.0, 95%CI:1.6-2.7, p < 0.001). Detectable TFV-DP was also more likely during pregnancy compared to postpartum (RR=1.4, 95%CI:1.1-1.7, p = 0.002) and among women aged ≥24 years compared to younger women (RR=1.8, 95%CI:1.3-2.6, p < 0.001).

**Conclusion:** Similar to studies of antiretroviral therapy among women living with HIV, we found that PrEP adherence was higher during pregnancy than postpartum, though adherence to 7 doses/week was low overall. Interventions should prioritize sustaining adherence in the postpartum period and increasing knowledge of partner HIV status, especially among younger women.
772 INCIDENT HIV INFECTIONS IN PREGNANT/BREASTFEEDING WOMEN AND INFANTS IN MOZAMBIQUE
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Background: According to UNAIDS, 40% of new pediatric infections in Mozambique are linked to incident maternal infection during pregnancy or breastfeeding. Since 2021, Mozambique has been implementing a policy of retesting all pregnant and breastfeeding women (PBFW) with unknown HIV status every three months until nine months postpartum. We aim to describe the provincial-level coverage of maternal retesting as well as the new policy was introduced and the association of incident maternal infections and positivity rates among HIV exposed infants

Methods: We analyzed the President’s Emergency Plan for AIDS Relief (PEPFAR) Monitoring, Evaluation, and Reporting data from April 1, 2021 to March 31, 2022. We report HIV prevalence at ANC1, the number of pregnant women who had HIV testing performed after first antenatal care visit (ANC1), maternal HIV retesting positivity and coverage (percent tested after ANC1/ HIV positive at ANC1) and early infant diagnosis (EID) positivity at 12 months by province. We assessed the association of maternal incident HIV infection and new pediatric infections, using a Pearson’s correlation coefficient

Results: Between April 2021 and March 2022, 99.3% (1,883,920, 1,896,554) of pregnant women attending antenatal care had their HIV status assessed at ANC1. Of these, 116,354 (6.2%) were positive, which included newly positive and known HIV positive. During this time period, 568,601 PBFW were tested after ANC1, achieving a retesting coverage of 32%. Retesting coverage was highest in Maputo City (112.6%), Maputo Province (92.8%) and below 60% in all other provinces. Positivity for HIV exposed infants ranged between 1.3% in Maputo City to 5.1% in Cabo Delgado. There was a significant positive correlation between retesting positivity at provincial level and EID positivity at 12 months (correlation coefficient 0.7, p-value 0.01) and a significant negative correlation between Post ANC1 coverage and 12 months’ EID positivity (correlation: -0.6, p-value 0.04) at provincial level

Conclusion: Higher provincial HIV positivity among retested PBFW, a measure of incident HIV infections during pregnancy or breastfeeding, was associated with higher HIV positivity among infants in Mozambique. Lower provincial HIV retesting coverage of PBFW was negatively correlated with higher infant HIV positivity suggesting that uptake of maternal retesting may be a proxy for uptake of critical PMTCT services. It is important to invest in preventing incident infections in PBFW through targeted interventions

Table 1 MER indicators by province

773 HIV RISK PERCEPTION AND PReP USE AMONG KENYAN WOMEN DURING AND AFTER PREGNANCY
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PrMMA Study
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Background: Self-perceived HIV risk influences PrEP use and may be altered during pregnancy and after delivery when women are motivated to protect their infants from HIV.

Methods: We analysed data from the PrMMA Study (NCT03070600), a cluster RCT that evaluated PrEP delivery models at 20 antenatal clinics in Western Kenya. Participants were enrolled and offered PrEP in pregnancy and followed through 9 months postpartum with HIV testing at each visit. A validated risk score developed to predict HIV acquisition among perinatal women defined high HIV risk (corresponding to 8.9 HIV infections per 100 person-years). HIV risk perception was assessed by asking “what is your gut feeling about how likely you are to get infected with HIV?” dichotomized as low (“extremely/very unlikely”) versus high (“extremely/very likely”).

Results: Among 2,429 women included in the analysis, median age was 24 years (IQR: 21–28), 82% were married, 22% did not know their partner’s HIV status, and 4% had a partner living with HIV. A quarter of women (27%) had high HIV risk during pregnancy, yet 57% of these women self-perceived low risk. Among women with high-risk (n=617), 194 (31%) women accepted PrEP. Women who perceived high-risk were more likely to have a partner known to be living with HIV (21% vs. 5%, prevalence ratio [PR]=1.5, 95% CI:1.2-2.2) and more likely to initiate PrEP (40% vs. 18%, PR=2.2, 95% CI:1.5-3.4). Among high-risk women, perceiving high HIV risk was associated with age >24 years (PR=1.4, 95%CI:1.0-1.9), prior pregnancy (PR=1.8, 95% CI:1.0-3.1), polygamous marriage (PR=1.6, 95% CI:1.2-2.2), and syphilis diagnoses in pregnancy (PR=2.6, 95% CI:1.5-4.4).

Conclusion: In this cohort, most high-risk women did not self-perceive high risk and most declined PrEP. Ensuring syphilis testing and improving knowledge of partner HIV status in PrEP delivery programs may refine risk perception and encourage PrEP uptake, particularly among younger women.

774 ANTEPARTUM WEIGHT GAIN AND ADVERSE PREGNANCY OUTCOMES: A MEDIATION ANALYSIS
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IMPAACT 2010
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Background: IMPAACT 2010 previously reported that low antepartum weight gain (< 0.18 kg/week) was associated with higher hazard of adverse pregnancy outcomes compared to normal weight gain (HR: 1.4, 95% CI: 1.04, 2.00), and that rates of adverse pregnancy outcomes differed by randomized ART arm. In this exploratory analysis, we evaluated whether change in antepartum weight was a mediator of by-arm differences in adverse pregnancy outcomes.

Methods: Women with HIV in 9 countries were randomized at 14-28 weeks gestational age (GA) to start dolutegravir (DTG)+emtricitabine (FTC)/tenofovir alafenamide fumarate (TAF) vs. DTG+FTC/tenofovir disoproxil fumarate (TDF) vs. efavirenz (EFV)/FTC/TDF. We defined a composite adverse pregnancy outcome as occurrence of stillbirth (GA ≥20 weeks), preterm delivery (GA < 37 weeks), or small for gestational age (SGA) < 10th percentile. Causal mediation analysis was used to separate the estimated effect of study arm on the risk of the composite pregnancy outcome into two effects: 1) effect mediated through the change in weight (indirect effect, modeled continuously), and 2) effect not mediated through the change in weight (direct effect, modeled as a binary outcome). We also performed a multivariable analysis adjusting for baseline GA, body mass index (BMI), CD4 count, country, and age.

Results: Of 443 participants, were randomized: 217 in DTG+FTC/TAF, 215 in DTG+FTC/TDF, and 211 in EFV/FTC/TDF arms. Baseline medians were: GA=21.9 weeks, HIV RNA 903 cp/mL, CD4 count 466 cells/μL, and BMI 26 kg/m². The proportion with an adverse pregnancy outcome was lower in the DTG+FTC/TAF arm (24%) compared to the EFV/FTC/TDF (32%) and DTG+FTC/TDF (33%) arms. Low weight gain was least common in the DTG+FTC/TAF arm (15%) compared to the DTG+FTC/TDF (30%) and DTG+FTC/TDF (24%). For comparisons with the DTG+FTC/TAF arm, the percent of risk of adverse pregnancy outcome
mediated by weight change was 31% for DTG+FTC/TAF vs EFV/FTC/TDF and 11% for DTG+FTC/TAF vs DTG+FTC/TDF. These results did not differ after adjustment (Figure).

Conclusion: In this population in which low antepartum weight gain was associated with a higher risk of adverse pregnancy outcomes, up to one-third of observed differences in adverse pregnancy outcomes between the randomized arms appear to be mediated by ART-related weight change. Further study is needed on the role of weight as well as additional ART-related mechanisms associated with these adverse outcomes in women living with HIV.

Mediation analysis of study arm, weight change, and the composite adverse pregnancy outcome*.

Table: Mediation analysis of study arm, weight change, and the composite adverse pregnancy outcome.*

HOMA at 1 mo among WLHIV in lasso regression. No maternal lipid subspecies were associated with infant HOMA in HIV- women.

Conclusion: In our cohort WLHIV showed partial compensation of pro-inflammatory eicosanoid mediator production. Compared to DTG, EFV was associated with maternal dyslipidemia in pregnancy which was predictive of lower infant insulin sensitivity. Further studies are warranted to delineate whether enhancement of anti-inflammatory mediator production may benefit maternal/infant metabolic outcomes.

Volcano plots comparing eicosanoid/lipid profiles in pregnancy between (A) WLHIV and HIV-seronegative women and (B) WLHIV on EFV vs. DTG.

776 CARDIOMETABOLIC RISK IN SOUTH AFRICAN POSTPARTUM WOMEN LIVING WITH HIV

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Background: Conflicting evidence suggests HIV and ART, including dolutegravir (DTG), increase the risk of cardiometabolic disease, but there is limited data in postpartum women living with HIV (WLH) or initiating DTG in pregnancy.

Methods: Among women living with HIV (WLH) and HIV- women enrolled in pregnancy and followed up to 26 months postpartum, cardiometabolic health was examined by HIV and by ART regimen (DTG vs efavirenz (EFV)) among post-conception initiators. Anthropometry (weight, height) and blood pressure (BP) were assessed by a trained study nurse using calibrated instruments. Body mass index (BMI) was calculated as weight divided by squared height and categorized per WHO guidelines. Postpartum weight change was calculated as weight measured postpartum minus self-reported pre-pregnancy. Participants underwent a 2-hour oral glucose tolerance test (OGTT) after an overnight fast. Lipids and insulin levels were also assessed in fasted samples.

Results: In 240 women (111 HIV+, 129 WLH), the median age was 30 (IQR, 26–35) and 15% were primigravid. Among WLH, 57% (n=73) initiated ART post-conception (73% [n=53] DTG; 27% [n=20] EFV); there were no differences in baseline characteristics by ART regimen. The median period for postpartum visit was 10 months (IQR, 7–12) and WLH came earlier than HIV- women (9 vs 10 months, p<0.01). Overall, high levels of postpartum obesity were observed in both WLH and HIV- women (52% vs 65%, p=0.01). Women on post-conception DTG had similar obesity levels as those on EFV (43% vs 55%, p=0.24). Most women gained rather than lose weight postpartum (3.4 kg IQR -2.6;9.0) overall. However, WLH experienced both high levels of postpartum weight gain (44%) and loss (45%). A similar trend of postpartum weight gain (36% vs 35%, p=0.62) and loss (57% vs 50%, p=0.62) was observed in both DTG and EFV groups. Overall, 18% of women had elevated BP, 23% stage 1 and 11% stage 2 hypertension, with no differences by HIV status and post-conception ART regimen. There were low levels of pre-diabetes overall and no differences by HIV status and post-conception ART regimen. However, WLH had lower LDL (2.07 mmol/L IQR, 1.95-2.19 vs 3.00 mmol/L IQR, 2.17-2.43, p<0.01) compared to HIV- women.

Conclusion: Postpartum weight gain, obesity and abnormal BP were common in WLH but were not related to DTG. Larger sample size studies are needed to confirm the impact of HIV and DTG on cardiometabolic health.
Immune Markers in Pregnant Women Living with HIV on ART therapy by Maternal BMI

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Background: Obesity impairs immune functioning so concerns exist that elevated body mass index (BMI) in HIV+ pregnant women could lead to immune dysregulation. This disruption of pregnancy associated immune processes could increase risk of pregnancy complications. We investigated temporal trends of inflammatory biomarkers and impact of maternal BMI.

Methods: We recruited a cohort of ART-eligible HIV+ pregnant women (n=521) making their first antenatal visit at a primary care facility in Cape Town, South Africa (April 2015-October 2016). Women were followed from their first antenatal visit through delivery with levels of inflammatory biomarkers (C-reactive protein, CRP; interferon gamma-induced protein 10, IP10; serum amyloid A, SAA) and maternal BMI measured at three visits (< 22 weeks, ~ 28 weeks and ~34 weeks) in pregnancy with an additional visit (2 weeks post-ART initiation) for women initiating antiretroviral therapy (ART) in pregnancy.

Analyses compared levels of biomarkers by timing of ART initiation (before vs during pregnancy) and by BMI status (normal < 24.9 kg/m², overweight 25-29.9 kg/m², obese ≥ 30 kg/m²).

Results: In the cohort (median age, 30y; 24% nulliparous); 47% (n=247) were on preconception ART (pre-ART) (84% TDF+XTC+EFV, 9% PI-based regimen) and the remaining 53% (n=274) initiated ART (79% TDF+XTC+EFV, 1% PI-based regimens). The majority of women in the cohort had an elevated BMI classified as obese (51%) or overweight (28%). Throughout pregnancy, CRP levels were relatively constant and no statistically significant differences were observed by ART status in normal, overweight or obese women, although obese women had lower SAA levels (p< 0.05). Among overweight women those initiating during pregnancy had higher levels throughout pregnancy. Women initiating during pregnancy had higher IP10 levels than their preconception ART counterparts, particularly among normal weight women. In the initiating women, IP10 levels declined following ART initiation (visit 1) across all BMI groups, while the levels appeared to increase in pregnancy in the women on preconception ART.

Conclusion: Differences observed in IP10 by ART status and BMI status are important findings IP10 has previously been implicated in the development of preeclampsia and which could impact pregnancy and birth outcomes.
778 VERTICAL TRANSMISSION IN INFANTS BORN TO WOMEN WITH HIV ON ANTIRETROVIRAL TREATMENT

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Background: Monitoring mother-infant pairs with HIV exposure is needed to assess effectiveness of vertical transmission prevention programmes and progress towards vertical transmission elimination.

Methods: We used routinely-collected data on infants with HIV exposure, born May 2018 – April 2021 to mothers diagnosed with HIV prior to delivery, in the Western Cape, South Africa, with follow-up through mid-August 2022. We assessed proportion of infants with HIV exposure who were diagnosed with HIV at birth (≤7 days), 10 weeks (>1 to 14 weeks) and >14 weeks as proxies for intrauterine, intrapartum/early breastfeeding and late breastfeeding transmission, respectively.

Results: Among 49,824 HIV-exposed infants, 925 (2%) were diagnosed with HIV. Among the mothers, 68% started antiretroviral treatment (ART) before and 27% during pregnancy; 90% received any ART during pregnancy and 86% received any ART in the year after delivery. Most pregnancy regimens included non-nucleoside reverse transcriptase inhibitors (84%); 11% integrase strand transfer inhibitors and 5% protease inhibitors. Of mothers with available results, 74% had viral load <100 copies/ml and 62% CD4 count ≥350 cells/µl at delivery. HIV-PCR test results were available for 83%, 67% and 48% of eligible infants at birth, 10 weeks and >14 weeks, respectively, and among infants with positive HIV-PCR tests, 47%, 18% and 35% were diagnosed at these respective time periods. Of infants who first tested positive at 10 weeks, 68% had previous negative birth tests (suggesting intrapartum/early breastfeeding transmission) and 52% who first tested positive at >14 weeks had previous negative tests at 1-14 weeks (suggesting late breastfeeding transmission). Of infants with HIV, 94% had mothers who started ART before or during pregnancy. Overall infant mortality was 1% (n=720/49,824) but was 4% (n=41/925) among infants with HIV. An additional 742 infants were diagnosed with HIV in the study period but were excluded as they were not diagnosed at 1 to 14 weeks.

Conclusion: Despite high maternal ART coverage, ongoing vertical transmission is a concern. Interventions to improve maternal viral suppression and reduce vertical transmission in pregnancy and breastfeeding are needed to achieve an HIV-free generation.

Vital status and HIV status at different time points for infants with HIV exposure

779 HIV DRUG RESISTANCE IN CASES OF PERINATAL TRANSMISSION IN IMPACTA 2010 (VESTED)

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IMPACTA 2010 Team

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Background: VESTED was a multicenter open-label, randomized phase III trial comparing the safety and efficacy of dolutegravir (DTG)- and efavirenz (EFV)-based regimens in pregnant and breastfeeding women. Perinatal transmission to the infant occurred in 4/617 (0.6%) mother-infant pairs. This sub-study evaluated HIV drug resistance (HIVDR) in these 4 mother-infant pairs.

Methods: Pregnant women with HIV between 14-28 weeks of gestation were randomized to initiate DTG+FTC/TAF, DTG+FTC/TDF, or EFV/FTC/TDF and followed through 50 weeks postpartum. HIVDR genotyping was performed on plasma (HIV RNA ≥200 cp/mL) or whole blood from study entry, infant diagnosis, and study exit. Single genome amplification (SGA) allowed assessment of minority variants in protease (PR), reverse transcriptase (RT), and integrase (IN) regions, and the nef 3’polypurine tract (3’PPT). SGA sequences were analyzed by Stanford HIVDR Database and 3’PPT was mapped to HIV-HXB2 to detect mutations.

Results: While awaiting study enrollment, all 4 women took EFV/3TC/TDF or EFV/FTC/TDF for 1-7 days; FIGURE. All infants breastfed and received nevirapine+/+zidovudine prophylaxis. Three cases of perinatal transmission occurred in mother-infant pairs randomized to DTG-ART and 1 to EFV-ART. A median of 18 viral templates (range 9-30) were sequenced for each HIV region. HIVDR to non-nucleoside RT inhibitors (NNRTIs) was selected (i.e., newly detected) in Mothers A and C. NNRTI HIVDR was detected in Infants A, B, and D at diagnosis; transmitted HIVDR potentially occurred in Infant A and was selected/acquired in B and D. IN, RT, and PR mutations were limited to those associated with low-level resistance except for M184V/I in 2 infants. 3’PPT A-to-G mutations were detected in 2 mothers at low frequencies.

Conclusion: High-level HIVDR to NNRTIs were observed. NNRTI mutations appear to have been acquired/selected in 2 mothers who switched from initial EFV- to DTG-based regimens likely due to the long half-life of EFV after excretion of other antivirals; and in 2 (possibly 3) infants from nevirapine prophylaxis (transmitted NNRTI resistance is possible in 1). While 3/4 cases of HIV transmission occurred from women randomized to DTG-ART, neither DTG HIVDR nor prevalent mutations in 3’PPT were detected. Our data show DTG-ART is associated with low rates of perinatal transmission and HIVDR; and similar to other studies, the majority of infants acquire NNRTI resistance; suggesting need infant prophylaxis regimens with a high barrier to HIVDR. Mother and infants’ plasma HIV RNA levels, genotypic resistance, and antiretroviral treatment
MATERNAL BROADLY NEUTRALIZING ANTIBODY ACTIVITY AND PERINATAL TRANSMISSION OF HIV-1

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

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

Results: Six out of 15 (40%) perinatal HIV-transmitting women from the MICS cohort neutralized over 50% of viruses of a heterologous, 10-virus global panel after correcting for non-specific neutralization activity (MLV). While this rate is higher than that reported in HIV-infected adults (20-30%), high neutralization breadth was also found among postnatal transmitting women with HIV-1 in the BAN and CHAVI cohorts (18 of 24, 75%) indicating that transmission during perinatal and postnatal settings may involve a similar high rate of maternal bNAb responses that could lead to viral escape.

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

RECONSTRUCTION OF THE ADCC ANTIBODY REPERTOIRE OF AN HIV-1 NON-TRANSMITTING MOTHER

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Background: Evidence from animal and human studies supports a role for Fc-mediated antibody (Ab) effector functions in protection from HIV acquisition and/or pathogenesis. In the setting of vertical transmission, passively-transferred Abs that mediate antibody-dependent cellular cytotoxicity (ADCC) correlate with reduced transmission, as well as improved clinical outcome in infants that acquire HIV. The characteristics of Abs that comprise the plasma ADCC response are not well understood. We isolated monoclonal Abs (mAbs) from an HIV non-transmitting mother to determine how these mAbs contribute to her plasma ADCC activity.

Methods: Memory B cells were sorted and cultured from a late pregnancy PBMC sample from Kenyan mother MG540, who did not transmit HIV to her infant during breastfeeding, despite high plasma and breastmilk viral loads. A targeted approach was used to identify wells with B cells producing HIV-specific Abs capable of ADCC. From these wells, mAbs were reconstructed and their epitopes defined using phage display or competition ELISA. ADCC was measured via the RFADCC assay. FcR-null (GRLR) variants were generated to determine how mAbs contribute to overall plasma ADCC activity.

Results: 16 mAbs, comprising 14 clonal lineages, were reconstructed. They all mediated ADCC and targeted various epitopes on the gp120 and gp41 subunits of HIV Envelope, including CD4-inducible epitopes recognized by prototypic mAbs A32, C11, and 17b, as well as the V3 loop and gp41 clusters I and II. Only the V3-specific mAbs were neutralizing, albeit weakly. In competition experiments, GRLR variants of gp120-specific mAbs individually reduced MG540 plasma ADCC by up to 10%, whereas combinations of 3-5 mAbs targeting distinct epitopes accounted for the majority of MG540 gp120-specific plasma ADCC (35%). GRLR variants of single gp41-specific mAbs accounted for up to 26% of gp41-specific plasma ADCC, with a combination of mAbs reducing plasma ADCC by 76%. GRLR variants of the MG540 mAbs recapitulated the passively-acquired ADCC activity of her infant, BGS490, to a similar degree.

Conclusion: The isolated MG540 mAbs collectively accounted for the majority of contemporary MG540 plasma ADCC and the passively-acquired ADCC activity of her infant, whereas individual mAbs contributed only a small percentage of the activity. These Abs targeted several epitopes across gp120 and gp41, indicating this was a highly polyclonal ADCC response. These mAbs also provide tools for further probing of plasma ADCC responses.

PBPK MODEL PREDICTION OF LONG-ACTING CAB AND RPV CONCENTRATIONS IN PREGNANCY

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Background: The pharmacokinetics (PK) of cabotegravir (CAB) (long-acting and rilpivirine (RPV) long-acting) has not been prospectively studied in pregnancy. Physiologically-based pharmacokinetic models (PBPK) offer the ability to incorporate physiologic pregnancy-associated changes and drug-specific characteristics to predict the concentration exposure during pregnancy.

Methods: We used a published maternal-fetal PBPK model by Dallmann et al. in Open Systems Pharmacology to predict concentrations of Cabotegravir (CAB) and Rilpivirine (RPV) after long-acting injection (LAI) in nonpregnant cisgender women during their 2nd and 3rd trimesters of pregnancy. This model was previously used to predict the maternal PK of Dolutegravir and Raltegravir and to verify the induction of UGT1A1 and CYP3A4 during the 2nd and 3rd trimesters. We obtained drug specific parameters for CAB and RPV from the published literature and used published clinical PK data for CAB and RPV (oral and IM) in nonpregnant, cisgender women to validate the nonpregnant PBPK model. We then extended the validated model to pregnancy and the PBPK model was utilized to simulate dose sequences in nonpregnant and pregnant individuals, including a loading dose paradigm, with monthly dosing up to 6 months.

Results: The simulation result using the maternal-fetal PBPK model is shown in Figure 1. According to the simulation results, the trough concentrations after the 1st injection were 29.5% and 23.0% lower during pregnancy compared to outside of pregnancy for CAB and RPV, respectively. The trough concentrations after the 4th injection were 31.1% and 29.2% decreased for CAB and RPV, respectively.
PHARMACOKINETICS AND VIROLOGIC OUTCOMES OF BICTEGRAVIR IN PREGNANCY AND POSTPARTUM

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IMPAACT 2026 Protocol Team
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Background: Bictegravir (BIC) is an HIV-1 integrase strand transfer inhibitor with potent antiviral activity. It is highly protein bound (~99.7%) and eliminated by UGT1A1 and CYP3A metabolism. Although protein binding and hepatic enzyme activity are altered during pregnancy, limited data exist on BIC pharmacokinetics (PK) in pregnancy. We describe preliminary BIC PK in pregnancy compared to postpartum and associated virologic outcomes.

Methods: IMPAACT 2026 is an ongoing, nonrandomized, open-label, parallel-group, multi-center phase-IV prospective study of antiretroviral PK in pregnant women with HIV. Intensive steady-state 24-hour PK sampling of oral BIC 50 mg once-daily (a component of Biktarvy®) and HIV viral load testing were performed during the 2nd and 3rd trimesters (2T, 3T), and 6-12 weeks postpartum (PP). Total BIC exposures were measured by a validated LC-MS/MS assay, quantitation limit of 0.078 mcg/mL. Geometric mean ratios (GMR) with 90% confidence intervals (CI) were calculated between 3T vs. PP.

Results: Preliminary analysis, triggered by low PK exposures, included 17 participants, 16 from the United States and 1 from Thailand (9 Black, 3 white, 1 Native American/Alaskan Native, 1 Asian and 3 unknown; 41% Hispanic/Latina; median entry age 31.0 years (interquartile range 25.8, 36.7)). BIC PK data were available for 6, 13, and 5 participants in 2T, 3T and PP, respectively. Compared with paired postpartum data (n=5), BIC AUC$_{\text{tau}}$ was 60% lower and C$_{\text{max}}$ was 53% lower in 3T (Table 1). All C$_{\text{max}}$ concentrations were above the estimated BIC protein-adjusted EC95 value of ~0.162 mcg/mL. Nine of 13 women had a 3T BIC AUC$_{\text{tau}}$ < the prespecified target, the 10th percentile for non-pregnant persons (58.7 mcg∙hr/ mL). Viral suppression (< 40 copies/mL) was achieved in 5/6, 12/13, and 4/5 participants at 2T, 3T and PP, respectively. The same participant had detectable viral loads at all three timepoints despite exceeding the 10th percentile BIC AUC$_{\text{tau}}$ for non-pregnant persons.

Conclusion: Total BIC exposures were lower during pregnancy compared to postpartum, yet all C24 concentrations were greater than the BIC EC95. Viral suppression was maintained in pregnancy and postpartum. As physiological changes during pregnancy can reduce drug protein binding, increased unbound BIC concentrations combined with the high potency of BIC may have contributed to observed viral suppression. Study enrollment is ongoing with collection of additional BIC PK results (including unbound concentrations), safety, and clinical outcomes.

### Table 1: Integrase Pharmacokinetic Parameters by Noncompartmental Analysis, Median (Q25-Q75)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
<th>Postpartum</th>
<th>GMR (lower CI - upper CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{\text{tau}}$ (mcg∙h/mL)</td>
<td>461 (210 - 1100)</td>
<td>425 (184 - 904)</td>
<td>124.5 (22.7 - 756.4)</td>
<td>0.45 (0.22 - 0.90)</td>
</tr>
<tr>
<td>C$_{\text{max}}$ (mcg/mL)</td>
<td>3.91 (2.75 - 4.02)</td>
<td>3.70 (2.34 - 4.03)</td>
<td>0.91 (0.58 - 1.43)</td>
<td>0.47 (0.27 - 0.80)</td>
</tr>
<tr>
<td>C$_{\text{max}}$(ng/mL)</td>
<td>0.75 (0.60 - 1.10)</td>
<td>0.59 (0.32 - 1.20)</td>
<td>0.22 (0.17 - 0.47)</td>
<td>0.30 (0.03 - 0.35)</td>
</tr>
<tr>
<td>V (L)</td>
<td>3.94 (2.31 - 3.82)</td>
<td>2.26 (1.15 - 3.24)</td>
<td>1.77 (0.85 - 3.92)</td>
<td>2.03 (1.16 - 3.52)</td>
</tr>
<tr>
<td>Cl (L/h)</td>
<td>54.83 (32.61 - 94.04)</td>
<td>30.04 (15.26 - 52.83)</td>
<td>17.17 (9.85 - 32.60)</td>
<td>2.03 (1.16 - 3.52)</td>
</tr>
</tbody>
</table>

**784** LONG-ACTING NANOFORMULATIONS REDUCE DOLUTEGRAVIR EXPOSURE TO EMBRYO BRAIN

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Background: Dolutegravir (DTG) is an integrase strand transfer inhibitor, which is currently recommended as part of first-line treatment regimens for HIV-1 patients worldwide. This is particularly due to its high potency, limited drug–drug interactions and intrinsic high genetic barrier to resistance. Nonetheless, concerns emerged for its usage in pregnant women. Notably, DTG-based regimens have been linked to neural tube defects and postnatal neurodevelopmental deficits when taken at conception. To this end, the need for intervention strategies that would maximize DTG’s efficacy benefits while limiting adverse events cannot be overstated. Thus, we created and evaluated an intramuscularly administered long-acting nanoformulated DTG (NDTG).

Our goal was to determine whether poloxamer coated NDTG would sustain therapeutic DTG levels in the mother’s peripheral system with minimal drug exposure to the embryo brain during pregnancy.

Methods: Female C3H/HeJ mice were either treated orally every day with DTG at human therapeutic equivalent dosage (5 mg/kg) starting at gestation day (GD) 0.5, with a single intramuscular (IM) injection of NDTG (45 mg/kg) at GD 0.5 or with two NDTG (25 mg/kg) intramuscular injections, first at GD 0.5 and second at GD 9.5. DTG concentrations were measured in plasma of dams and in whole brain tissues of embryos at GD 16.5 and at GD 17.5, respectively, using mass spectrometry (LC-MS/MS).

Results: Single (45 mg/kg) or two (25 mg/kg) IM injections of NDTG achieved equivalent plasma DTG levels to daily oral DTG administration (5 mg/kg) in pregnant dams. In all the treatment groups, plasma DTG levels were between 4000–6500 ng/mL, which are comparable to therapeutic DTG concentrations from daily oral dosing in humans. However, significantly lower levels of DTG biodistribution in embryo brain was observed following NDTG injections in comparison to daily oral administration. For daily oral DTG administration, average DTG concentrations of 196 ng/g were recorded in the embryo brains compared to 34 ng/g and 45 ng/g for groups administered with single or two IM injections of NDTG, respectively.

Conclusion: This work demonstrates that long-acting DTG nanoformulations sustain therapeutic DTG levels in maternal plasma while limiting drug exposure to the embryo brain during pregnancy. Our preliminary work shows that novel drug delivery approaches could potentially minimize embryo brain DTG exposure and thus, potentially limit drug related neurodevelopmental toxicities.
DAPIVIRINE RING SAFETY AND DRUG DETECTION IN BREASTFEEDING MOTHER-INFANT PAIRS

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Background: Global guidance supports exclusive breastfeeding for infants up to six months of age, continued breastfeeding with introduction of complementary foods. World Health Organization (WHO) guidance also supports provision of oral pre-exposure prophylaxis (PrEP) for breastfeeding people at substantial risk of HIV acquisition. In January 2021, WHO recommended the 25 mg dapivirine vaginal ring (DVR) as an HIV prevention product. Drug concentrations are presented in the Table. Results indicate high TDF and FTC concentrations from infant DBS were all below the lower limit of quantitation.

Methods: Microbicide Trials Network (MTN)-043 was a phase 3b, randomized, open-label trial, with 12 weeks of exposure to monthly DVR or daily oral PrEP (200 mg emtricitabine [FTC]/300mg tenofovir disoproxil fumarate [TDF]). From September 2020 to July 2021, healthy, HIV-negative, exclusively breastfeeding, mother-infant pairs were enrolled 6-12 weeks after delivery at sites in Malawi, South Africa, Uganda, and Zimbabwe. Mother-infant pairs were randomized in a 3:1 ratio (DVR: PrEP). Adverse event data were collected for mothers and infants throughout product exposure and at two weeks following end of product use. Drug concentrations were measured in maternal plasma, maternal dried blood spots (DBS), breast milk, infant plasma, and infant DBS.

Results: We enrolled 197 mother-infant pairs (DVR: 148, PrEP: 49). Median infant age at enrollment was 9 weeks and >95% of visits were completed. No SAEs or ≥Grade 3 events in mothers or infants were deemed related to study product. Drug concentrations are presented in the Table. Results indicate high uptake of study product in both arms with extremely low concentrations of tenofovir diphosphate concentrations from infant DBS were all below the lower limit of quantitation.

Conclusion: In this first evaluation of DVR safety and drug detection during breastfeeding, few SAEs or ≥Grade 3 AEs occurred among mothers and infants and all infant AEs were deemed unrelated to study product. While dapivirine appears to concentrate in breastmilk, detection in infant plasma was low. This favorable safety profile, along with data demonstrating low dapivirine exposure per TFV-DP levels, including pregnancy loss, pre-term birth or small for gestational age. Overall, 12% of women with TFV-DP had adverse pregnancy or birth outcomes compared to 11% in women without any TFV-DP in their 2nd or 3rd trimester (OR=0.98; 95%CI=0.67-1.42).

PREGNANCY AND BIRTH OUTCOMES FOLLOWING ORAL PrEP USE BY OBJECTIVE LEVELS OF TDF/FTC

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Background: TDF and FTC have been evaluated for safety among pregnant and lactating people taking PrEP. Prior safety studies measured exposure using self-reported recent adherence, which may over- or under-report actual use. This is one of the first studies to compare pregnancy and birth outcomes using objective levels of tenofovir disoproxil fumarate (TDF-DP) in dried blood spots (DBS) of women in the 2nd or 3rd trimester of pregnancy.

Methods: We enrolled pregnant women >15 years old without HIV at first antenatal care visit and offered them PrEP following HIV counseling. We quantified TDF-DP levels in DBS in pregnant women who reported taking PrEP in the past 30 days in the 2nd or 3rd trimester. We used logistic regression models with generalized estimating equations to evaluate pregnancy and birth outcomes by TFV-DP (any vs. none), adjusting for age, gestational age and gravidity.

Results: In 300 pregnant women, median age was 26 yrs (IQR=23-32yrs); gestation age at baseline was 16 wks (IQR=12-21wks). Overall, 7.3% had TFV-DP concentrations corresponding to >2 doses/wk (n=22); 27.3% of levels corresponded to 2-6 doses/wk (n=82); 27.3% of levels corresponded to <2 doses/wk (n=82); 38% had levels below the limit of quantification (BLQ; n=114). Correlates of having TFV-DP concentrations corresponding >2 doses/wk included older age, higher education and condomless sex in past month. Comparing women with any TFV-DP in DBS (n=186; 62%) vs. those with no TFV-DP (n=114; 38%), 97% had live births (adjusted odds ratio [aOR] for any TFV-DP in DBS vs. BLQ = 1.35; 95%CI=1.05-4.02). There were no differences in pregnancy or birth outcomes by TFV-DP level (Table). The composite adverse pregnancy and birth outcome included pregnancy loss (miscarriage, stillbirth), neonatal death, pre-term (<37wks gestation) and infants small for gestational age. Overall, 12% of women with TFV-DP had an adverse pregnancy or birth outcome, compared to 11% in women without any TFV-DP in their 2nd or 3rd trimester (OR=0.98; 95%CI=0.67-1.42).

Conclusion: Our study is one of the first to compare objective PrEP use with birth outcomes. Pregnancy and birth outcomes did not differ by PrEP exposure per TFV-DP levels, including pregnancy loss, pre-term birth or small for gestational age. Our study provides further evidence that TDF is safe in pregnancy and highlights the importance of counseling women on the effective use and safety of TDF/FTC as PrEP in pregnancy. Limitation includes the differing background for TFV-DP collection in pregnancy.
787 ANGIOGENIC BIOMARKERS OF POOR OUTCOMES IN PREGNANT WOMEN WITH HIV IN BOTSWANA
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Background: The Botswana-based Tshilo Dikotla study identified associations between dolutegravir (DTG)-containing antiretroviral therapy (ART) regimens initiated prior to conception and maternal hypertensive (HTN) and infant outcomes. This study examined the role of angiogenic biomarkers in maternal plasma samples collected from mothers delivering between 26-28 weeks gestation. The primary aim was to assess the association between maternal plasma levels of angiopoietin 2 (Ang2), placental growth factor (PlGF), and soluble Fms like tyrosine kinase 1 (sFlt-1) with adverse pregnancy outcomes. The secondary aim was to determine whether any differences existed between dolutegravir (DTG)-containing ART and non-DTG-containing ART regimens.

Methods: Women with HIV were on DTG/TDF/FTC. Levels of angiopoietin 2 (Ang2), placental growth factor (PlGF), and soluble Fms like tyrosine kinase 1 (sFlt-1) were quantified by enzyme-linked immunosorbent assay. Differences in log-transformed values between groups were compared using Student's t-test. PlGF levels and sFlt-1:PlGF ratios were assessed using standard cut-offs, where a level <12pg/ml or a value >85 respectively is indicative of a high-risk pregnancy. Proportions of women below or above cut-offs, as applicable, were compared by maternal HIV status using Chi-squared testing. Logistic regression models were fit to assess associations of each biomarker with maternal HTN and infant SGA (<10th percentile), stratified by HIV status and adjusting for maternal BMI.

Results: Log-transformed Ang2, PlGF, and sFlt-1 levels did not differ significantly between groups. Compared to women without HIV, a higher proportion of women with HIV had levels of PlGF <12pg/ml and a sFlt-1:PlGF ratio >85 (PlGF: 17.4% vs. 1.5%, p=0.002; sFlt-1:PlGF ratio: 19.3% vs. 2.9%, p=0.0036). PlGF below and sFlt-1:PlGF ratio above cut-offs were significantly associated with maternal HTN at 26-28 weeks (PlGF: aOR 11.2 [2.4–51]; sFlt-1:PlGF aOR 7.8 [1.8–33]) and with infants born SGA (PlGF: adjusted odds ratio 1.72 [1.04–2.88]; sFlt-1:PlGF ratio: adjusted odds ratio 1.90 [1.05–3.45]). Women with HIV were significantly more likely.

Conclusion: Angiogenic biomarkers known to be associated with placental dysfunction and adverse pregnancy outcomes, sFlt-1 and PlGF, were altered in women living with HIV and living with HIV on DTG-based ART. This was indicative of a high-risk pregnancy and was associated with poor pregnancy outcomes to detect and intervene on pregnancies at elevated risk of poor fetal growth.

788 DOLUTEGRAVIR EXPOSURE AND CONGENITAL ANOMALIES IN SUB-SAHARAN AFRICA
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Background: The Botswana-based Tshilo Dikotla study enrolled pregnant women, those with and without HIV, between 16-36 weeks gestation and followed their infants. This analysis included 114 women (46 with HIV) with maternal plasma samples collected between 26-28 weeks gestation. Women with HIV were on DTG/TDF/FTC. Levels of angiopoietin 2 (Ang2), placental growth factor (PlGF), and soluble Fms like tyrosine kinase 1 (sFlt-1) were quantified by enzyme-linked immunosorbent assay. Differences in log-transformed values between groups were compared using Student's t-test. PlGF levels and sFlt-1:PlGF ratios were assessed using standard cut-offs, where a level <12 pg/ml or a value >85 respectively is indicative of a high-risk pregnancy. Proportions of women below or above cut-offs, as applicable, were compared by maternal HIV status using Chi-squared testing. Logistic regression models were fit to assess associations of each biomarker with maternal HTN and infant SGA (<10th percentile), stratified by HIV status and adjusting for maternal BMI.

Results: Log-transformed Ang2, PlGF, and sFlt-1 levels did not differ significantly between groups. Compared to women without HIV, a higher proportion of women with HIV had levels of PlGF <12pg/ml and a sFlt-1:PlGF ratio >85 (PlGF: 17.4% vs. 1.5%, p=0.002; sFlt-1:PlGF ratio: 19.3% vs. 2.9%, p=0.003)). PlGF below and sFlt-1:PlGF ratio above cut-offs were significantly associated with maternal HTN at 26-28 weeks (PlGF: aOR 11.2 [2.4–51]; sFlt-1:PlGF aOR 7.8 [1.8–33]) and with infants born SGA (PlGF: adjusted odds ratio 1.72 [1.04–2.88]; sFlt-1:PlGF ratio: adjusted odds ratio 1.90 [1.05–3.45]). Women with HIV were significantly more likely.

Conclusion: Angiogenic biomarkers known to be associated with placental dysfunction and adverse pregnancy outcomes, sFlt-1 and PlGF, were altered in women living with HIV and living with HIV on DTG-based ART. This was indicative of a high-risk pregnancy and was associated with poor pregnancy outcomes to detect and intervene on pregnancies at elevated risk of poor fetal growth.
ALI-7 were categorized into quartiles for risk assessment. We estimated time-to-event curves and hazard ratios associated with ALI quartiles and employed robust errors to account for the case-cohort design.

**Results:** From 2017-2019, 800 women were enrolled in the IPOP trial; 51 (6%) delivered spontaneously before term (cases). We randomly selected 107 participants for inclusion in the sub-cohort, 6 of whom were cases. We then selected all remaining cases of spontaneous preterm birth (n=45), yielding a final case-cohort population of 512. Z-score distributions of systolic blood pressure, maternal heart rate, high-density lipoprotein, triglycerides, hemoglobin A1C, albumin, and 25-OH Vitamin D met criteria for inclusion in the subset ALI. ALI-15 was not associated with spontaneous preterm birth. However, in time-to-event analysis between quartiles of ALI-7, participants in the fourth quartile had higher hazard of the primary outcome (HR 2.28, 95% CI 1.04-4.97) compared to those in the second quartile.

**Conclusion:** High allostatic load index among pregnant women with HIV was associated with spontaneous preterm birth. Early detection and preventive intervention among high-risk women with HIV could have a substantial public health benefit.

Figure 1. Incidence curves of spontaneous preterm birth over time by z-score quartile of allostatic load index

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791 VAGINAL INFLAMMATORY MARKERS ASSOCIATED WITH PRETERM BIRTH IN ZAMBIAN WOMEN WITH HIV

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**Background:** HIV infection is a known risk factor for adverse pregnancy outcomes, including preterm birth (PTB). There is a limited understanding of the maternal biological mechanisms that result in PTB, and how those mechanisms are impacted by HIV. We performed a targeted proteome analysis of vaginal samples to determine the association between HIV and preterm birth in Zambian pregnant women.

**Methods:** We conducted a case-cohort study that consisted of 160 Zambian women with and 136 women without HIV, of which 51 or 30, respectively, experienced PTB outcomes. Dry vaginal swabs collected at < 24 and 28 weeks of gestational age (GA) were subjected to a targeted proteomic analysis consisting of 17 key inflammatory, angiogenic, and immune biomarkers IL-1α, IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL-10, IL-12, IFNγ, IFNα2, TNFα, sTNF R1, and CXCL10 were measured using Abcam FirePlex-384 assays. sCD14 and LBP were measured using Luminex multiplex assays, and i-FABP was measured by ELSA. Concentrations were log-transformed before the results were analyzed using an unpaired t-test with Welch’s correction using Graphpad Prism 9.

**Results:** At both timepoints, women with HIV who had term deliveries presented with higher levels of inflammatory mediators, including TNF-α, IL-12, IL-23 and sCD14, whereas vaginal IFN-g levels were lower compared to women without HIV (p values ranging from p<0.05 to p<0.0001). Compared to women with term birth, women without HIV and PTB had higher levels of IL-12 (p<0.01), IL-23 (p<0.01), and IFN-α2 (p<0.05) at both visits, whereas women with HIV had increased vaginal levels of IL-6 (p<0.02) and IL-1β (p<0.02) at 24 or 28 wks GA, respectively. Among all PTB cases, women with HIV had increased vaginal concentrations of sCD14 (p<0.03) and IFAB (p<0.05) and higher levels of IL-1β (p<0.01) at both timepoints. In contrast, IL-4 (p<0.05), IL-10 (p<0.03), and IFNα2 (p<0.01) were lower at 24 wks GA compared to women without HIV and remained lower at >28wks GA.

**Conclusion:** Even in pregnancy progression to term birth, the vaginal milieu in women with HIV is characterized by heightened inflammation compared to women without HIV. Vaginal inflammation was a common factor for PTB in women with and without HIV. However, among all PTB cases, increased levels of IL-1β and markers of microbial translocation appear to be associated with PTB and distinguish women with HIV from women without HIV.
investigated the impact of participant selection, data source and data quality on observed associations between maternal ART and PTD.

**Methods:** Data from 3 related data sources from the same underlying population over same time-period (January 2017-July 2018) in Western Cape Province (South Africa) were compared. 1) Province-wide routine electronic health data on pregnant women (records linked across health services by the Provincial Health Data Centre (PHDC)); 2) facility-based pregnancy exposure registry (PER) (based on digitisation of patient-held booklet recording pregnancy-related clinical information); and 3) a purposely sampled cohort study (questionnaire-based data collection and clinical records abstraction).

Data availability, detail and quality of baseline characteristics varied by data source. Associations between maternal ART and PTD were examined separately to assess impact of data source on the association. We compared PTD by HIV status (HIV+ vs HIV-) and ART status (initiation preconception vs during pregnancy).

**Results:** Median age (27y) was similar across data sources, however proportions of primigravid and HIV+ women differed: PHDC (n=183593), 53% primigravid, 19% HIV+ of whom 56% on ART preconception; and cohort study (n=989) 21% primigravid, 48% HIV+ of whom 70% on ART preconception (82% TDF+FTC+EFV, 7% PI-based regimens). Among women with live singleton births, PTD deliveries differed by HIV status/ART status in PHDC (population-level); differences were less pronounced in PER and cohort study (Figure).

Increased PTD risk in HIV+ women (ARR1.15, 95% CI 1.11-1.18) and women on preconception ART (ARR1.08, 95% CI 1.01-1.14) was only observed in PHDC. Data availability and quality did not impact association estimates, however statistically significant differences by HIV/ART status were only detectable in the larger PHDC population.

**Conclusion:** With improvements in birth outcomes due to increased coverage of safer ART regimens, sample size considerations will be important to detect statistically significant differences by ART status. Inclusion of both population level data and purposely sampled studies will remain important to leverage the benefits of both data sources.

![Preterm delivery by HIV/ART Status](Image 104x303 to 245x397)

793 **PREGNANCY HORMONAL DYSREGULATION CORRELATES WITH HIV-EXPOSED INFANT GROWTH OUTCOMES**

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**Background:** Many hormones in pregnancy regulate fetal growth. Few data exist on fetal growth-related hormones in pregnancy in women living with HIV (WLHIV) and whether maternal hormonal alterations are associated with infant anthropometrics. **Methods:** The Tshilo Dikotla study prospectively enrolled pregnant women in Botswana. WLHIV receiving dolutegravir/tenofovir/emtricitabine and HIV- seronegative (HIV-) women were included in this analysis. Levels of estradiol (E2), sex-hormone binding globulin (SHBG), cortisol, growth hormone 1 (GH1), insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 1 (IGFBP-1) were measured by ELSIA in plasma collected between 24-28 weeks gestation. Bioavailable E2 (bE2) was calculated using measured values of E2 and SHBG. Infant anthropometrics were converted to weight-for-age and length-for-age Z-scores (WAZ, LAZ) at birth and 1 year-of-life. Data was normalised by log transformation. Generalized linear models were fit to evaluate associations between each hormone and 1) HIV status (model #1), 2) infant anthropometrics at birth and 12 months (model #2). The anthropometrics model included an interaction term between HIV status and each hormone to assess effect modification by HIV status.

**Results:** Plasma specimens were available from 114 women (46 WLHIV), of which 95 had a live birth while in the study. WLHIV were older (27 vs. 26 years), had higher gravidity (3 vs 1), and were less likely to be breastfeeding (78% vs. 100%) than HIV- women. Among WLHIV, median enrollment CD4 count was 494 cells/mm³, and 90% had HIV RNA levels < 40 copies/ml at enrollment. After adjusting for maternal age, BMI, and gestational age of specimen draw, WLHIV had lower mean log bE2 (β: -0.22, p=0.028), cortisol (β: -0.22, p=0.001), and IGF1 (β: -0.81, p=0.007), but higher GH1 (β: 0.91, p=0.011) (Fig 1). Log bE2 was positively associated with birth WAZ (β: 0.91, p=0.011) and log IGF1 (β: 0.40, p=0.004) with infant WAZ at 12 months after adjusting for maternal age and BMI, duration of exclusive breastfeeding, and birth WAZ.

**Conclusion:** Growth hormone levels are dysregulated in WLHIV suggestive of impaired placentia function. The dysregulation, particularly of GH1 and IGF1, may influence growth in the first year of life among infants exposed to HIV.

![Figure 1](Image 362x421 to 536x538)

794 **SARS-CoV-2 ANTIBODY RESPONSES POST-INFECTION IN PREGNANCY BY VACCINATION STATUS**

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**Background:** We evaluated SARS-CoV-2 antibody binding and neutralization responses at delivery among pregnant persons with prior SARS-CoV-2 infection by vaccine status. **Methods:** We enrolled participants with evidence of prior SARS-CoV-2 infection detected in pregnancy (anti-nucleocapsid (anti-N) IgG+ on enrollment or prior RT-PCR+ or antigen+) and followed them through delivery. Maternal delivery and cord blood samples were tested for SARS-CoV-2 binding antibodies to spike (anti-S) (from vaccination and/or infection) and anti-N (from infection only) IgG by Abbott Architect followed by neutralizing antibodies (classified as neutralizing if serum dilution inhibited infection by 50% [ND50 heat] ≥20 and R2 ≥0.9) if sample volume allowed. Positive IgG thresholds were Abbott index ≥1.4 for anti-N and ≥50 AU/ml for anti-S. Chi-squared test was used to compare differences in proportions between groups. Wilcoxon rank sum test was used to compare medians.

**Results:** Among 71 participants with delivery and cord samples, median age was 33 years (interquartile range [IQR] 30-35) and median gestational age was 31.7 weeks (IQR 18.0~37.9) at enrollment in pregnancy. By delivery, 17 (24%) participants were unvaccinated, 21 (30%) were partially vaccinated or had completed a primary series, and 33 (46%) were boosted. Median time from infection (RT-PCR+ or antigen+ result) to delivery was 16.7 weeks (IQR 9.7-24.3). At delivery, 33 (46%) of maternal (median 3.2 index) and 37 (52%) of cord samples (median 3.1 index) were anti-N IgG+.

Participants with ≥1 vaccine were more likely to be anti-S IgG+ than those unvaccinated (100% vs. 82%, p < 0.01), have higher median anti-S IgG+ (25,000 vs 1,019 AU/ml, p < 0.01), and have neutralizing antibodies (100% vs. 81%, p < 0.01) with higher median log₉ neutralization (14.00 vs 12.41, p < 0.01) at delivery.

![Figure 2](Image 104x303 to 245x397)
median anti-S IgG+ (25,000 vs 1,188 AU/ml, p<0.01), and have neutralizing antibodies (100% vs. 75%, p<0.01) with higher median log_{10} neutralization (14.00 vs 12.41, p<0.01) at delivery.

**Conclusion:** Among pregnant people with prior SARS-CoV-2 infection detected during pregnancy, maternal and cord blood antibody binding and neutralization responses were higher among those receiving SARS-CoV-2 vaccination prior to delivery.

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**SARS-CoV-2 SEROPREVALENCE TREND AMONG PREGNANT WOMEN IN ZAMBIA, 2021-2022**

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**Background:** Confirmed COVID-19 case counts underestimate SARS-CoV-2 infections, particularly in countries with limited testing capacity. Pregnant women attending antenatal care (ANC) clinics have served as healthy population surrogates to monitor diseases like HIV and malaria. We measured SARS-CoV-2 seroprevalence among women attending ANC clinics to assess infection trends over time in Zambia.

**Methods:** We conducted repeated cross-sectional surveys among pregnant women aged 15–49 years attending their first ANC visits in 3 districts of Zambia during September 2021-September 2022. Up to 200 women per district were enrolled each month, completing a standardized questionnaire. Dried blood spot samples were collected for serologic testing using the Tetracore® FlexImmArray™ SARS-CoV-2 Human IgG Antibody Test and HIV testing according to national guidelines. Dried blood spot samples were collected for serologic testing using the Tetracore® FlexImmArray™ SARS-CoV-2 Human IgG Antibody Test, a multiplex assay targeting SARS-CoV-2 spike and nucleocapsid proteins. We calculated age-adjusted odds ratios (aORs) for SARS-CoV-2 seroprevalence by demographic characteristics, adjusting for the district.

**Results:** A total of 5,351 women were enrolled at 29 study sites between September 2021 and September 2022. Participants’ median age was 25 years (interquartile range: 21–30), 530 (9.9%) tested positive for HIV, and 101 (1.9%) reported a prior positive COVID-19 test. Overall, SARS-CoV-2 seroprevalence was 67%, and rose from 49% in September 2021 to 85% in September 2022 (Figure 1). The greatest increase in seroprevalence occurred during the 4th wave caused by the Omicron variant (48% in December 2021 to 63% in January 2022). Seroprevalence was significantly higher among women living in urban districts (Chipata and Lusaka) compared to rural Chongwe District (Chipata OR: 1.2, 95% confidence interval (CI): 1.1-1.4; Lusaka OR: 1.7, 95% CI: 1.5-2.0). The age group was not significantly associated with seroprevalence after adjusting for the district (aOR: 1.1, 95% CI: 1.0-1.2). Seroprevalence was significantly lower among pregnant women living with HIV than women living without HIV (aOR: 0.8, 95% CI: 0.6-0.9).

**Conclusion:** Overall, two-thirds of women in the three surveyed districts in Zambia had evidence of SARS-CoV-2 exposure, rising to 85% after the Omicron variant spread throughout the country. ANC clinics have a potential role in ongoing SARS-CoV-2 serosurveillance and can continue to provide insights into SARS-CoV-2 infection dynamics. Furthermore, they provide a platform for focused SARS-CoV-2 prevention messaging and COVID-19 management in pregnant women at higher risk of severe disease.
COVID-19 VACCINATED MATERNAL/CORD BLOOD DON'T HAVE NEUTRALIZATION FOR OMICRON VARIANTS

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Background: Maternally derived antibodies are crucial for neonatal immunity. Understanding the binding and -cross neutralization capacity of maternal/cord antibody responses to COVID-19 vaccination during pregnancy can inform neonatal immunity.

Methods: Here we characterized binding and neutralizing antibody profile in delivery of 24 pregnant individuals following two doses of Moderna mRNA-1273 or Pfizer BNT162b2 vaccination. We evaluated the translational antibody transfer by profiling maternal and umbilical cord blood. We analyzed for SARS-CoV-2 multivalent cross-neutralizing antibody levels for wildtype Wuhan, Delta, Omicron B1, B2, and B4A/B4A5 variants by enzyme-linked immunosorbent assay

Results: Our results reveal that current vaccination induced significantly higher (p=0.003) RBD-specific binding IgG titer in cord blood compared to maternal blood for both Wuhan and Omicron B1 strain. Interestingly, binding IgG antibody levels for the Omicron B1 strain were significantly lower (P<0.0001) when compared to the Wuhan strain in both maternal and cord blood. In contrast to the binding, the Omicron B1, B2, B4A/B4A5 specific neutralizing antibody levels were significantly lower (P<0.0001) compared to the Wuhan and Delta variants. It is interesting to note that the B4A/B4A5 neutralizing capacity was not at all detected in both maternal and cord blood.

Conclusion: Our data suggest that the initial series of COVID-19 mRNA vaccines were immunogenic in pregnant women, and vaccine-elicted binding antibodies were detectable in cord blood at significantly higher levels for Wuhan and Delta variants but not for Omicron variants. Interestingly, the vaccination did not induce neutralizing antibodies for Omicron variants. These results provide novel insight into the impact of vaccination on maternal humoral immune response and translational antibody transfer for SARS-CoV-2 variants and support the need for boosters as new variants emerge.

HIV-EXPOSED UNINFECTED (HEU) INFANTS DISPLAY UNIQUE PRO-INFLAMMATORY BIOPROFILES

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Background: HEU infants are at risk for adverse metabolic, infectious, and neurodevelopmental outcomes when compared to HIV unexposed/uninfected (HUU) infants. However, the relationships between maternal immunity and transplacental antibody transfer for SARS-CoV-2 variants and support the insight into the impact of vaccination on maternal humoral immune response and transplacental antibody transfer for SARS-CoV-2 variants and support the need for boosters as new variants emerge.

Methods: Here we characterized binding and neutralizing antibody profile in delivery of 24 pregnant individuals following two doses of Moderna mRNA-1273 or Pfizer BNT162b2 vaccination. We evaluated the translational antibody transfer by profiling maternal and umbilical cord blood. We analyzed for SARS-CoV-2 multivalent cross-neutralizing antibody levels for wildtype Wuhan, Delta, Omicron B1, B2, B4A/B4A5 specific neutralizing antibody levels were significantly lower (P<0.0001) compared to the Wuhan and Delta variants. It is interesting to note that the B4A/B4A5 neutralizing capacity was not at all detected in both maternal and cord blood.

Conclusion: Our data suggest that the initial series of COVID-19 mRNA vaccines were immunogenic in pregnant women, and vaccine-elcited binding antibodies were detectable in cord blood at significantly higher levels for Wuhan and Delta variants but not for Omicron variants. Interestingly, the vaccination did not induce neutralizing antibodies for Omicron variants. These results provide novel insight into the impact of vaccination on maternal humoral immune response and transplacental antibody transfer for SARS-CoV-2 variants and support the need for boosters as new variants emerge.
Conclusion: We found no evidence that the breast milk microbiome is altered by ART in lactating women living with HIV compared with women LWH and not on ART.

RBC FOLATE CONCENTRATIONS IN MOTHERS/INFANTS RANDOMIZED IN PREGNANCY: DTG VS EFV
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Background: The Botswana Tsepamo Surveillance study found a possible association between dolutegravir (DTG) use at conception and increased risk of neural tube defects. To explore mechanisms of this early finding, we hypothesized that DTG may block cellular uptake of folate.

Methods: We conducted a substudy in IMPAACT 2010, a multicenter, open-label, randomized controlled phase 3 clinical trial that assigned pregnant women between 14-28 weeks of gestational age to initiate one of three antiretroviral treatment (ART) regimens: DTG + emtricitabine (FTC)/tenofovir alafenamide (TAF), DTG + FTC/tenofovir disoproxil fumarate (TDF), or efavirenz (EFV)/FTC/TDF. Red blood cell (RBC) folate concentrations, normalized for hematocrit, were assessed at study entry and delivery in mothers and at birth in infants. Outcomes were: 1) maternal RBC folate from entry to delivery, 2) infant RBC folate and 3) ratio of infant-to-maternal RBC folate at birth/delivery.

Generalized estimating equation models for the log e of folate outcomes were fit to estimate the geometric mean ratio (GMR) and 95% confidence intervals (CIs) of each arm comparison, unadjusted and adjusted for precision variables. The estimated GMR trajectory of maternal RBC folate was compared between arms with a ratio (GMR/C).

Results: 340 mothers had at least one RBC folate measurement available: 114 in each DTG arm and 112 in the EFV arm. 310 infants had a folate measurement. Median maternal age was 25 years (IQR 22, 30) with the majority from Africa (78%). Median CD4 count was 482 cells/mm^3 and median log10 HIV RNA was 3 copies/ml. At study entry, median gestational age was 22 weeks (IQR 17, 25). Overall, 90% of mothers received folic acid supplements and 90% lived in countries with folic acid fortification of food. RBC folate concentrations at entry were similar across arms. The estimated geometric mean trajectory of maternal folate was only 3% higher in the DTG + FTC/TAF arm than the EFV/FTC/TDF arm (aGMR/T: 1.03, 95% CI 1.00, 1.06). The DTG + FTC/TAF arm had only an estimated 8% lower infant-maternal folate ratio (aGMR 0.92, 95% CI 0.78, 1.09) compared to the EFV/FTC/TDF arm. Results are consistent with no clinically meaningful differences between treatment arms in maternal RBC folate trajectory, infant RBC folate, or infant-to-maternal RBC folate ratio at birth/delivery.

Conclusion: Our findings suggest that cellular uptake of folate and transport of folate to the infant do not differ in pregnant persons starting DTG vs EFV based ART (nor TAF vs TDF).
802 IMPACT OF IN-UTERO EXPOSURE TO HIV AND LATENT TB ON INFANT HUMORAL RESPONSES

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Background: Latent tuberculosis (LTBI) is a common coinfection in people with HIV. How maternal HIV and LTBI influence the development of an infant’s immune response is not well characterized. We hypothesized that maternal antibodies (Ab) may interact with the infants’ immune responses to TB and BCG providing the basis for this study.

Methods: Frozen plasma from pregnant women with HIV (14-34 weeks gestation) (IGRA- vs IGRA+ vs ATB) and infants (−/− vs −/+ vs +/+) were evaluated for Ab against TB and BCG based on antepartum IGRA status (IGRA- vs IGRA+ vs ATB). No significant differences were found between IGRA- and IGRA+ mothers. Mothers who developed ATB at delivery were evaluated at the time of diagnosis and had higher PPD, ESA6/CFP10, and AgSSA IgG (p = 0.006, p = 0.03, p = 0.007), ESA6/CFP10 and Lipoarabinomannan (LAM) IgM (p = 0.04, p = 0.05), and PPD and ESA6/CFP10 IgG (p = 0.01). Infants (−/+ vs −/−) showed no differences in TB-specific plasma Ab responses at 12 weeks. Infants (+/- vs -/-) exhibited a trend for lower IgG against LAM compared to -/- infants at 44 weeks.

Results: Maternal plasma from entry was evaluated for Ab based on antepartum IGRA status (IGRA- vs IGRA+ vs ATB). No significant differences were found between IGRA- and IGRA+ mothers. Mothers who developed ATB at delivery were evaluated at the time of diagnosis and had higher PPD, ESA6/CFP10, and AgSSA IgG (p = 0.006, p = 0.03, p = 0.007), ESA6/CFP10 and Lipoarabinomannan (LAM) IgM (p = 0.04, p = 0.05), and PPD and ESA6/CFP10 IgG (p = 0.01). Infants (−/+ vs −/−) showed no differences in TB-specific plasma Ab responses at 12 weeks. Infants (+/- vs -/-) exhibited a trend for lower IgG against LAM compared to -/- infants at 44 weeks. IGRA+ infants exhibited a trend for higher PPD (p = 0.066) and AgSSA-specific LAM IgM (p = 0.049, p = 0.055), and PPD and ESAT6/CFP10, and AgSSA IgG (p = 0.006, p = 0.03, p = 0.007), and AgSSA-specific IgG (p = 0.091) IgG at 44 weeks compared to IGRA- infants.

Conclusion: Exposure to maternal LTBI in utero does not significantly differentiate the infant’s Ab profile against TB and BCG, however, we observed a trend for reduced LAM-specific IgG responses at 44 weeks in (+/-) infants. Further evaluation of the function of these Ab and cellular immunity to BCG would provide greater insight into the effect HIV and TB exposure in utero has on the infants’ responses to BCG and protection from TB.

IGRA- Infant Antibody Responses

Table 1: Logistic regression models of factors associated with lower academic performance in children completing standard 3 or 4.

<table>
<thead>
<tr>
<th>Coefficients of Interest</th>
<th>Unadjusted Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Human Exposure to HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-exposed</td>
<td>2.31 (1.05, 5.11)</td>
<td>0.04</td>
</tr>
<tr>
<td>Low Maternal Education¹</td>
<td>0.30 (0.03, 2.80)</td>
<td>0.31</td>
</tr>
<tr>
<td>Maternal Education/Health</td>
<td>1.04 (0.97, 1.12)</td>
<td>0.33</td>
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<td>Maternal Income¹</td>
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<tr>
<td>&lt;$10,000/mo</td>
<td>0.99 (0.80, 1.20)</td>
<td>0.90</td>
</tr>
<tr>
<td>Household Food Insecurity</td>
<td>1.00 (0.76, 1.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>Presence of HIV²</td>
<td>1.00 (0.76, 1.30)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note: Maternal antiretroviral therapy was included in the adjusted model.

803 LOWER ACADEMIC PERFORMANCE AMONG CHILDREN WITH PERINATAL HIV EXPOSURE IN BOTSWANA

Kathleen M. Powis, Lesedi Lebanna, Sara Schenkel, Gosego Masasa, Samuel W. Kgoile, Martha Ngwacca, Paige L. Williams, Amy L. Slogrove, Roger L. Shapiro, Shahn Lockman, Mompati O. Mmalane, Josef Mkhabela, Jennifer Jao, Adam R. Cassidy


Background: Higher risk of suboptimal neurodevelopment has been identified among children who are HIV-exposed but uninfected (HEU) compared to children born to women without HIV in some studies. However, academic performance of school-aged children by HIV exposure status has not been well studied.

Methods: The Botswana-based FLORISH study is an ongoing prospective observational study re-enrolling mother-child pairs who previously participated in maternal-child health studies through the Botswana Harvard AIDS Institute Partnership and for which data on maternal HIV status, antiretroviral treatment (ART), obstetric history, and child HIV status and outcomes through at least 18 months-of-life had been prospectively collected. FLORISH parents report their child’s past school grades at enrollment. A Cochran-Mantel-Haenszel test was used to compare academic performance between children who are HEU vs. HIV-unexposed uninfected (HUU) and whose last grade completed was standard 3 or 4 (4th grade) and 5th grade United States equivalence. Lower academic performance was defined as an overall grade for all coursework of “C” or lower (<55%). Unadjusted and adjusted logistic regression models were fit to identify factors associated with lower academic performance.

Results: Of 160 children, 114 were HEU. 16 (14%) children HEU were born preterm (<37 weeks gestation) compared to 4 (9%) children HUU. Among children HEU, 76% were exposed to utero to triple ART, 23% to only zidovudine, and 1% had no fetal antiretroviral exposure. Women with HIV were more often older at enrollment, a higher proportion had no primary education only (16% versus 0%), and less likely to have breastfed (19% vs 100%). A higher proportion of children HEU had lower academic performance compared to their HUU peers (71% vs 48%; p = 0.013). In adjusted analyses, children HEU remained significantly more likely to have lower academic performance (Adjusted odds ratio: 2.31 [95% Confidence Interval: 1.05, 5.11]; p = 0.04) (Table 1).

Conclusion: In this small cohort in Botswana, primary school academic performance was lower among children HEU compared to children HUU. If confirmed, this could have significant human capital implications for countries, such as Botswana, where >20% of infants are born HEU. Identifying modifiable contributors is of paramount importance, as it is the development of screening tools to identify children at risk of poor academic achievement and interventions to mitigate the risk well before initiation of formal education.

804 PSYCHIATRIC DISORDERS IN HIV-EXPOSED UNINFECTED VS NON-HIV-EXPOSED CHILDREN

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Background: Psychiatric health of HIV exposed uninfected (HEU) children in the era of maternal antiretroviral therapy (ART) remain unclear. We aimed to...
compare risk of psychiatric disorders among HEU children to a matched control group of HIV unexposed uninfected (HUU) children, born in Denmark.

Methods: In a nationwide register-based study we included all HEU children born in Denmark, 2000-2020. Each HEU child was individually matched by year of birth, maternal age at birth, and maternal immigration status to 10 HUU children. The primary outcome was risk of any psychiatric disorder (ICD-10 F00-F99). Incidence rate ratios were estimated using Poisson regression, both univariate and multivariate analysis adjusting for child and maternal confounders. Person-years at risk (PY) were calculated from birth until onset of outcome (incident diagnosis), emigration, death, or end of follow-up (December 31st, 2020). Analyses stratifying by age and sex were also conducted.

Results: In total, 550 HEU children and 5,500 HUU children were included. HIV-infected mothers were more likely to be of African origin (54% vs. 9%) and their infants were more likely to be born preterm (<37 weeks) (12% vs 6%) and to be delivered by Caesarean Section (65% vs. 27%). At the time of delivery, all HIV-infected mothers were on ART and 87% had HIV RNA levels <50 copies/ml. HEU children had an increased risk of any psychiatric disorder (IRR 1.45; 95% CI: 1.04 - 2.04) in the unadjusted analysis, but in the adjusted analysis, the risk was only significant for children aged 6-11 years (aIRR 1.85; 95% CI: 1.06 - 3.23) (Figure 1). Stratifying by sex, girls aged 6-11 years had an increased risk of any psychiatric disorder (aIRR 4.40; 95% CI: 1.71 - 11.36), while boys had an increased risk at age 12-20 years (aIRR 2.58; 95% CI: 1.13 - 5.90). Compared to HUU girls, HEU girls had an increased risk of anxiety (aIRR 4.00; 95% CI: 1.67 - 9.69) which was also the first common psychiatric disorder among HEU girls (n=10). The most common psychiatric disorder among HEU boys (n=8) was attention-deficit hyperactivity disorder (ADHD). However, there was no difference in risk of ADHD between the HEU and HUU boys (aIRR 0.61; 95% CI: 0.20 - 1.86).

Conclusion: In a high-resource setting, HEU children had an increased risk of any psychiatric disorder compared to HUU children, especially among the 6-11 years-old girls and the 12-20 years-old boys. These findings highlight the importance of addressing the mental health needs of HEU children and young adults.

Figure 1. Time to first diagnosis of any psychiatric disorder among HIV-exposed uninfected (HEU) children and HIV unexposed uninfected children (HUU) boys and girls aged 6-11 years (aIRR 1.85; 95% CI: 1.06 - 3.23) (Figure 1). Stratifying by sex, girls aged 6-11 years had an increased risk of any psychiatric disorder (aIRR 4.40; 95% CI: 1.71 - 11.36), while boys had an increased risk at age 12-20 years (aIRR 2.58; 95% CI: 1.13 - 5.90). Compared to HUU girls, HEU girls had an increased risk of anxiety (aIRR 4.00; 95% CI: 1.67 - 9.69) which was also the first common psychiatric disorder among HEU girls (n=10). The most common psychiatric disorder among HEU boys (n=8) was attention-deficit hyperactivity disorder (ADHD). However, there was no difference in risk of ADHD between the HEU and HUU boys (aIRR 0.61; 95% CI: 0.20 - 1.86).

Background: Many factors influence pubertal onset including in utero exposure to medications and infections. Few studies have evaluated the influence of in utero HIV/antiretroviral exposure on pubertal onset in children who are HIV-exposed and uninfected (CHEU).

Methods: CHEU in the Surveillance Monitoring for ART Toxicities study of the Pediatric HIV/AIDS Cohort Study and children who are HIV-unexposed/uninfected (CHUU) in the Bone Mineral Density Cohort with sexual maturity rating (SMR) assessments at age 9yr (±4mo) were included. Puberty was defined as SMR >2 separately by sex assigned at birth (girls: breasts and/or pubic hair; boys: genitalia and/or pubic hair). Weights were used to standardize sex and racial distribution of CHUU to CHEU. Log-binomial regression models were fit to estimate the adjusted relative risks (RR) for puberty at age 9 in CHEU vs. CHUU. Among CHEU, log-binomial regression models were fit to assess the association of puberty at age 9 with: 1) protease inhibitor (PI) exposure at ≤30wk gestation, 2) maternal CD4 ≤200 vs. >200 cells/mm³, 3) HIV viral load (VL) copies/ml, and 4) child body mass index (BMI) >95th percentile.

Results: 227 CHEU (113 boys, 114 girls) and 344 CHUU (162 boys, 182 girls) were included. CHEU boys were more likely to have reached puberty by age 9 compared to CHUU boys (15% vs. 9.9% genitalia, 11.2% vs. 3.7% pubic hair, 10.3% vs. 5.2% both), with an estimated 2-fold higher (RR=2.07, 95%CI 0.89, 4.79) risk for genitalia and 3.5 fold higher risk (RR=3.55, 95%CI 0.92, 13.81) for pubic hair. Among girls, the prevalence of puberty at age 9 in CHEU and CHUU was 35.2% vs 23.1% for breasts, 32.4% vs 14.8% for pubic hair, 27.6% vs 8.8% for both) with similar risks for breast (RR=0.96, 95%CI 0.65, 1.41) and pubic hair (RR=1.10, 95%CI 0.70, 1.72). Among CHEU, 74% were exposed to PI at ≤30wk gestation. Factors associated with CHEU having begun puberty by age 9 differed by sex (Table). Among boys, those with PI exposure at ≤30wk gestation had a lower risk of reaching puberty by age 9 for genitalia and pubic hair, while higher maternal VL in pregnancy increased the risk of both outcomes. For girls, high BMI was associated with a higher prevalence of SMR >2 breasts.

Conclusion: Male CHEU were more likely to have reached puberty by age 9 compared to CHUU. Higher maternal viremia in pregnancy was associated with higher risk and PI exposure with lower risk of presence of puberty at age 9 in males, but not in females. Further confirmatory and mechanistic studies are warranted.

Table: Adjusted associations of PI exposure, CD4 count, viral load in pregnancy, and child BMI with sexual maturity rating (SMR) greater than or equal to 2 for each indicator by sex for CHEU.

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**Table**: Adjusted associations of PI exposure, CD4 count, viral load in pregnancy, and child BMI with sexual maturity rating (SMR) greater than or equal to 2 for each indicator by sex for CHEU.

**Results**:

- **PI exposure**: Male CHEU had a lower risk of presence of puberty at age 9 for genitalia and pubic hair, while higher maternal VL in pregnancy increased the risk of both outcomes. For girls, high BMI was associated with a higher prevalence of SMR >2 breasts.

**Conclusion**:

- Male CHEU were more likely to have reached puberty by age 9 compared to CHUU. Higher maternal viremia in pregnancy was associated with higher risk and PI exposure with lower risk of presence of puberty at age 9 in males, but not in females. Further confirmatory and mechanistic studies are warranted.

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**Methods**:

- **CHEU in the Surveillance Monitoring for ART Toxicities study**:)
- **CHUU in the Bone Mineral Density Cohort**:)

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**Background**:

- Many factors influence pubertal onset including in utero exposure to medications and infections. Few studies have evaluated the influence of in utero HIV/antiretroviral exposure on pubertal onset in children who are HIV-exposed and uninfected (CHEU).

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- **CHEU in the Surveillance Monitoring for ART Toxicities study**:)
- **CHUU in the Bone Mineral Density Cohort**:)

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**Table**: Adjusted associations of PI exposure, CD4 count, viral load in pregnancy, and child BMI with sexual maturity rating (SMR) greater than or equal to 2 for each indicator by sex for CHEU.
806 MORTALITY LINKED TO HIGHER INFLAMMATION IN PERINATALLY-INFECTED HIV+ KIDS
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EPICAL Consortium
1Bambino Gesu Children’s Hospital, Rome, Italy, 2Hospital Universitario de Ourense, Orense, Spain, 3University of Miami, Miami, FL, USA, 4University of Padova, Padova, Italy, 5Boston Children’s Hospital, Boston, MA, USA

Background: Mechanisms underlying mortality in some early ART-treated HIV-perinatally infected children remain unclear. The prospective EART study (Early Anti-Retroviral treatment in HIV-infected children), enrolled in 6 African centers, 220 perinatally HIV-infected children collecting clinical data and peripheral blood (<2 weeks of age). Trying to identify biomarkers predictive of mortality risk at enrollment visit, we assessed the differential plasma concentration of pro-inflammatory molecules in 3 different groups: deceased (dead-HIV+), non-deceased HIV+ children (HIV+) and healthy control (HC) study participants.

Methods: Selection of patients was done using Nearest Neighbor matching 1:2. A propensity score distance was used to select 59 individuals (HIV+) within the EART cohort, with similar characteristics at entry (including CD4 count, HIV viremia, weight for height, ART adherence, age at enrollment, age at ART start, country of birth, and prematurity) to 20 deceased children. Plasma samples were analyzed using an Inflammation panel proximity extension assay (Qlink platform; T96) and benchmarked to 13 HC HIV unexposed controls. Principal Component Analysis (PCA) was done to visualize patterns in proteomics.

Results: PCA analysis on proteomic data revealed a gradient in the distribution of samples with dead-HIV+ clustering separately from HC and matched HIV+ participants (Fig.1A). Top 15 loading proteins highlighted molecules as pro-inflammatory IL-6, CXCL11 (potential agonist of CCR3, involved in activated T cells chemotaxis) and CCL7 (also known as MCP-3, important for the activation and chemotraction of macrophages) as death driving proteins (Fig.1B). Differential analysis at the same time point, also revealed significant higher levels in the relative concentration (NPX) of these 3 molecules, with the plasma concentration within PCA.

Conclusion: HIV+ children who prematurely died demonstrated higher concentrations of inflammatory molecules at enrollment, suggesting a possible role of inflammation in driving eventual mortality. Larger studies are needed to confirm early molecular correlates of mortality in HIV+ children which may identify actionable biomarkers and inform trials of novel immunomodulatory drugs to reduce mortality.

Figure 1. A) PCA showing the sample distribution among the groups: HIV+, dead-HIV+ and HC; B) Top 15 loading proteins contributing to samples distribution within PCA.

807 INFECTED NAIVE CD4+ T CELLS IN CHILDREN WITH HIV CAN Proliferate AND Persist on ART
Mary Grace Katusiime1, Shuang Guo2, Victoria Neer3, Sean C. Patro4, Xiaolin Wu4, Anna Horner5, Ann Chahroud6, Maud Magviron7, Mary F. Kearney2
1Fred Hutchinson Cancer Center, Seattle, WA, USA, 2National Cancer Institute, Frederick, MD, USA, 3Fred Hutchinson National Laboratory for Cancer Research, Frederick, MD, USA, 4Emory University, Atlanta, GA, USA, 5Emory Vaccine Center, Atlanta, GA, USA

Background: We previously showed that HIV persists in perinatally infected children through clonal expansion of T cells infected before the initiation of ART. Although HIV primarily infects memory CD4+ T cells (TMem), recent studies in adults and SIV/HIV-infected rhesus macaques have shown that naive CD4+ T cells (TNaive) harbor a higher proportion of intact HIV genomes. Little is known about the TNaive HIV reservoir in early treated children. The aims of this study were to determine (i) if HIV infects TNaive in early-treated children, (ii) the infection frequency relative to TMem, (iii) the proportion that are predicted intact, and (iv) if infected TNaive can undergo cellular proliferation to form infected T cell clones.

Methods: The cohort consisted of 8 children aged 5-11 years who initiated ART at a median of 4 weeks of age (range 0-39) with suppressed viremia for a median of 8.5 years. PBMC were sorted into TMem (CD45RO-CD28 + CD27+CDS-CCR7+CDSRA+) and TNaive (CD45RO+ CDS5+). Multiple displacement amplification (MDA) was performed on genomic DNA from 320,000 TNaive and 106,667 TMem from each child and dispensed in 96-well plates at limiting dilution for HIV proviruses. To detect MDA wells containing an HIV provirus and to estimate the proportion of the HIV+ MDA wells with predicted intact proviruses, probe-based PCR methods were used to screen for HIV LTR, Psi, and RRE. Integration site analysis (ISA) was performed on TNaive in the child with the highest TNaive, frequency of infection.

Results: Integration site analysis resulted in purities of a median 96.6% (range 95-100%) for TMem and 97% (range 96.5–100%) for TNaive. HIV-infected TNaive were detected in all 8 children at a median of 37.5% infected cells/million (range 6-231), a mean of 11-fold lower than infected TMem in the same children. Of the 201 HIV LTR+ TNaive detected in the 8 children, 4 were predicted intact (6.5% of proviruses with detectable Psi and/or RRE, 2% of LTR+). ISA identified 8 clones of infected cells in the TNaive subset. None of 8 infected cell clones were found to carry intact HIV proviruses.

Conclusion: We found that infected TNaive persist in children with perinatal HIV on ART for 5-11 years. Some infected TNaive can proliferate into clones of infected cells. Measurements adapted from the Intact Provirus Detection Assay, showed that 6.5% of infected TNaive (2% of LTR+) are predicted to be intact. Our results demonstrate that TNaive are an important HIV reservoir in perinatally infected children on ART.

808 LONGITUDINAL T CELL IMMUNE PROFILING IN HIV-EXPOSED INFECTED AND UNINFECTED INFANTS
Lesley de Armas1, Sureesh Pallikkuth2, Stefano Rinaldi3, Vinh Dinh3, Paula Vaz3, Nadia Sito3, Nicola Cotugno4, Rajendra Pahwa1, Paolo Palma4, Maria Grazia Lapin4, Savita Pahwa1
1University of Miami, Miami, FL, USA, 2Fundação Aries Glaser Contra o SIDA Pediátrico, Maputo, Mozambique, 3Instituto Nacional de Saúde, Maputo, Mozambique, 4Bambino Gesu Children’s Hospital, Rome, Italy

Background: In perinatal HIV infection, early ART initiation is now recommended but questions remain regarding infant immune responses to HIV as well as the impact of HIV on early immune development.

Methods: We conducted a longitudinal study in HIV exposed infected (HEI,n=33) and uninfected (HEU,n=35) infants from Maputo, Mozambique (TARA cohort). Although ART was prescribed at diagnosis (1-2mo), adherence was inconsistent with only 6/33 achieving suppression within 5 mo of starting ART and maintaining plasma virus load (VL) < 200 copies/ml for 2 yrs. T cell phenotypes were assessed at ages 1-2 mo. (entry, Pre-ART), 5, 10, and 18 mo. Expression of markers of activation and immune regulation (CCR5, CD127, CD25, CD28, CD31, CD38, CXCR5, HLA-DR, PD-1, TIGIT) were measured on CD4 and CD8 T cell subsets by flow cytometry. Median VL in HEI at entry was 5.7 logs (range 2.5-7 logs). Spearman correlation was performed to find associations between T cell phenotypic data and VL.

Results: Compared to HEU at entry, higher frequencies of cells expressing activation markers, HLA-DR, PD-1, CD38, and CD25 and lower frequencies of CD28 and CD127 (IL-7R) were noted in CD4 and CD8 T cells of HEI. At entry, CD127+ CD4 T central memory cells correlated positively with VL (r=0.53, p=0.002) and CXCR5+ CD4 T cells correlated negatively with VL (r=-0.54, p=0.001). Dynamic changes in surface receptor expression were evident from 1 to 18 mo. in HEI and HEU infants, with CXCR5, PD-1, and TIGIT increasing and CD127 and CD38 decreasing with age in CD4 and CD8 T cells. Thus, to identify age-independent immunological correlates of VL in HEI at different timepoints longitudinally, we excluded T cell parameters that showed significant correlations (p<0.05) with all three of the following: age in HEU, age in HEI, and VL in HEI leaving 62/382 parameters (16%). of these, co-expression of HLA-DR and CD38 on CD8 T cells was a strong positive indicator of plasma VL levels (r=0.44, p< 0.0001).
Conclusion: Our results point to a role for IL7/IL7R signaling in early response to HIV infection in infants and suggests that the magnitude of IL-7 receptor downregulation on memory CD4 T cells in response to IL-7 levels could be a correlate of viral control. Further, we report the T cell developmental changes directly attributed to HIV burden and provide a strategy for identifying age-independent correlates of viral control in infants that may be important to guide the design of immune-based interventions, including vaccines, in early life.

Relationship of CD127 (IL7R) expression on CD4 T cells and Virus Load in Infants

809 TORQUE TENOVIRUS: MARKER OF IMMUNE RECONSTITUTION IN PERINATALLY HIV-INFECTED INFANTS

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1Hospital General Universitario Gregorio Marañón, Madrid, Spain, 2La Paz University Hospital, Madrid, Spain, 3Hospital General Universitario de Castellón, Castellón de la Plana, Spain, 4Hospital Universitario Virgen del Rocío, Sevilla, Spain, 5Hospital Materno Infantil Las Palmas, Las Palmas de Gran Canaria, Spain, 6Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, 7Hospital Donostia, San Sebastián, Spain, 8University Hospital Gregorio Maranón, Madrid, Spain

Background: Torque Teno Virus (TTV), a small, circular, single-stranded DNA virus, is an integral part of the human virome, whose implications in terms of immune response are barely understood. Recently, studies have suggested its potential use as an immunological marker in immunocompromised patients. The aim of this study was to measure TTV viral load in a cohort of perinatally HIV-infected patients (PHIV) and explore its association with immune reconstitution.

Methods: Chronic PHIV on stable antiretroviral treatment (ART) with and without undetectable HIV viral load were selected from the Spanish Cohort of PHIV (CoRISpe/FARO), and compared to a cohort of uninfected controls. Plasma samples and peripheral blood mononuclear cells (PBMCs) were obtained from the Spanish HIV BioBank. Plasma TTV detection and quantification was assessed by qPCR and T-cell phenotype was studied by multiparametric flow cytometry on PBMCs. Correlations with baseline CD4 and CD8 and long-term immune and HIV VL evolution were analysed.

Results: A total of 57 PHIV (44% males) were included and compared to 23 HIV-uninfected healthy donors (34% males) (HD). At baseline, PHIV were younger (20 [17-24] vs 26 [24-27] years; p = 0.001). Their median CD4 T-cells was 736 [574-906] and had a median of 17 years [14-20.5] since ART initiation and 65 months [39-116] under virological control. TTV viral load in plasma was significantly higher among PHIV (Fig. A) and in males compared to females (p = 0.02). TTV viral load correlated with CD4 and CD8 T-cell and the CD4/CD8 ratio (p = 0.002; r = -0.39, p = 0.037; r = -0.277, p = 0.005; r = 0.37 respectively) among PHIV (Fig. B-D), but not with CD4 nadir, age at ART initiation or time under HIV suppression. Among PHIV, TTV viral load positively correlated with the co-expression of HLA-DR/CD38 in CD4 T-cells (r = 0.01, r = 0.39) and the soluble proinflammatory biomarker IL-6 (p = 0.04, r = 0.37). Baseline TTV viral load was higher in patients who lost HIV suppression during the follow-up (p < 0.05). After three and five years of follow-up, changes in CD4/CD8 ratio from baseline time-point, inversely correlated with TTV levels (p = 0.09; r = -0.33 and p = 0.06; r = -0.56 respectively).

Conclusion: TTV viral load was significantly higher among PHIV. Despite associations with T-cell activation and IL-6 were mild, TTV viral load strongly correlated with the CD4/CD8 ratio, suggesting its potential value as an immunological predictor.
MORTALITY RISK AMONG CHILDREN <5 YEARS OLD LIVING WITH HIV ON ART

Mortality risk among those on ART is less clear. We aimed to describe and compare mortality risk among CLHIV <5 yo on ART with that of older people living with HIV (PLHIV) ≥5 yo on ART.

Methods: We analyzed US President’s Emergency Plan for AIDS relief (PEPFAR) Monitoring, Evaluation, and Reporting data collected quarterly from all PEPFAR-supported sites globally during October 2019–March 2022. We described the total number of deaths reported, proportion of those on ART who died (number of reported deaths in the current reporting quarter divided by the sum of PLHIV on ART in the previous reporting quarter and PLHIV started on ART in the current reporting quarter), and viral load suppression rates (VLS: proportion of PLHIV on ART with VL result reported who were virally suppressed [HIV RNA <1000 copies/mL]). Crude mortality ratios (CMR) were calculated comparing proportion who died among CLHIV <5 yo (stratified by <1 and 1–4 yo to differentiate infants from other children <5 yo) and PLHIV ≥5 yo (stratified by 5–14, 15–49, and ≥50 yo) on ART during Oct 2019–Mar 2022. Results: From Oct 2019–Mar 2022, on average 123,636 CLHIV <5 yo were on ART each quarter (n=14,152 <1 yo and n=109,484 1–4 yo); in total 7,949 CLHIV <5 yo on ART died during Oct 2019–Mar 2022. CMR comparing proportion who died among children <5 years old (yo) living on ART with viral load suppression treatment (ART) and older people ≥5 yo living with HIV on ART during October 2019–March 2022 in all supported sites.

RELATIVE GAP IN HIV VIRAL LOAD SUPPRESSION IN YOUNGER CHILDREN IN ETHIOPIA

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Background: Children living with HIV (CLHIV) <5 years old (yo) not on antiretroviral treatment (ART) experience disproportionately high mortality. Mortality risk among those on ART is less clear. We aimed to describe and compare mortality risk among CLHIV <5 yo on ART with that of older people living with HIV (PLHIV) ≥5 yo on ART.

Methods: We used aggregate national VL data from Data for Accountability, Transparency, and Impact (DATIM) for 2019-2022. Annual trend analysis of VL (number tested/total eligible on ART) and VLS (number suppressed/number tested) are presented. Percentage is computed to describe the trend in VLS and VLS and the absolute number of VL unsuppressed (>1,000 copies/mL) cases is also presented.

Results: At baseline (July 2019-June 2020), the VLS in the age group 1-4 and 5-9 years was in the lowest range, 77% and 77% respectively. The highest baseline VLS (95%) was reported among the age group 45-49 and 50+. The baseline VL was relatively lower in the age group 1-4 (46%), 5-9 (66%), 20-24 (64%) and 25-29 (66%) while the VLS in the remaining age groups at baseline was above 70%. After two years, VLS in age group 1-4 and 5-9 showed a relative improvement (84% and 92% respectively) while all other age bands beyond 25 years exceeded the 95% target. The two years result showed that VLS increased modestly among younger children: 56% and 72% for 1-4- and 5–9-year-old respectively. In July 2021-June 2022, the only adult age groups with VLS less than 80% were 20–24 and 25–29 corresponding with their relatively lower baseline result. A total of 24,042 unsuppressed cases were reported in July 2019-June 2020 most of which (88.4%) were in the adult age group above 15 years of age. The number of unsuppressed patients significantly reduced over the three years in all age groups and reached at 10,739 in July 2021-June 2022 (reduced by 55.3% from the baseline) and the adult proportion was 92.9% with relative increase from the baseline.

Conclusion: Ethiopia is on track in achieving the UNAIDS third 95 target within all age groups in 2030 provided the VLS performance gap among younger children is addressed. Improving VLS among children and young adults is beneficial to distinctly understand their VL suppression status. Understanding the root causes of low VLS and VLS among children on ART and devising targeted interventions is very important. The progressive reduction in the absolute number of unsuppressed cases is an encouraging result.
Trend in viral load coverage and viral load suppression among patients on ART in Ethiopia, July 2019 – June 2022

813 FANMI: A RANDOMIZED TRIAL OF COMMUNITY COHORT HIV CARE FOR ADOLESCENT GIRLS, HAITI
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Background: Adolescent girls and young women are the epicenter of the global HIV epidemic and in need of multilevel interventions to improve their health outcomes.
Methods: FANMI, a randomized-controlled trial, evaluated the effectiveness of community-based cohort HIV care versus standard of care (SOC) among adolescent and young adults living with HIV (AYALH) in Haiti. Females, 16-24 years who were newly diagnosed with HIV at clinic or community HIV testing sites, or defaulted >6 months from care, were randomized 1:1 to FANMI vs SOC. FANMI was designed to improve convenience, social support and stigma by grouping AYALH in cohorts of 6-10 peers to attend monthly HIV care sessions in a community center with integrated clinical care, group counseling, and social activities led by the same provider. National guideline changes during the study included switching participants to dolutegravir regimens and expanding SOC visits to 6 months. The primary outcome was 12-month retention defined as any visit 9-15 months from enrollment. Secondary outcomes included viral suppression (<1000 copies/ml), risk behaviors, and acceptability using interviews.
Results: 120 AYALH enrolled (60 per arm) between May 2018-January 2021. Median age was 21, 91% were newly diagnosed, and median CD4 count was 591 cells/mm3 (IQR 399-788). A total of 78.3% (47/60) FANMI participants vs 85.0% (51/60) in SOC achieved the primary outcome (unadjusted RR=0.92 95% CI 0.78-1.09, p=0.35). Excluding 9 participants who never attended a FANMI/SOC visit after enrollment, 12-month retention was 88.7% (47/53) in FANMI vs 87.9% (51/58) in SOC (RR =1.01 95%CI 0.88-1.15, p=0.90). Participants who presented for HIV testing vs community testing and achieved the primary outcome: 95% vs 70% (FANMI) and 83% vs 88% (SOC). Viral suppression among those retained at 12 months: 44.6% (21/47) in FANMI and 37.3% (19/51) in SOC (RR =1.20 95%CI 0.74-1.9, p=0.43). There were no differences in pregnancy and risk behaviors. Providers preferred FANMI reporting increased time for counseling and peer support. FANMI participants reported high acceptability, decreased stigma, and increased social support with no confidentiality breaches. Limitations included interrupted study operations during the COVID-19 pandemic.
Conclusion: FANMI was not more effective for AYALH in Haiti but was preferred by providers and highly acceptable to participants. It offers promise as a complementary program for high-risk AYALH in low-income settings facing barriers to clinic-based care.

814 ECONOMIC STRENGTHENING INTERVENTION TO IMPROVE ART ADHERENCE IN HIV-INFECTED YOUTH
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Background: Adolescents living with HIV (ALHIV) have low antiretroviral therapy (ART) adherence and poor treatment outcomes. Poverty is a major threat to adherence, yet many interventions do not address poverty to improve adherence. In this cluster-randomized trial, we examined the impact of a multi-component asset-based economic empowerment intervention on ART adherence among ALHIV in Uganda.
Methods: In this study, we enrolled 702 ALWHIV aged 12-16 from 39 clinics (intervention=20, control=19) in Uganda between January 2014 and December 2015. Thirty-nine clinics were randomized into the control (n=344) or intervention arm (n=358). The intervention consisted of a long-term child development account (CDA), four micro-enterprise workshops, and 12 mentorship and educational sessions. Adherence was measured using unannounced pill counts, where good adherence was defined as taking 85% of prescribed pills. We used mixed-effects logistic regression analysis to examine the effect of the intervention on ART adherence, while controlling for clustering at the clinic. Study is registered at ClinicalTrials.gov (#NCT01790373).
Results: The mean age was 12.6 years, and 58% were females. Intervention effect: The intervention significantly improved ART adherence, OR=1.99 (95% CI: 1.09 – 2.96). Main effect of time: adherence gradually declines at every visit (except visit two); third visit OR=0.40 (95% CI: 0.26 – 0.64); fourth visit OR=0.35 (95% CI: 0.24 – 0.52); fifth visit OR=0.31 (95% CI: 0.22 – 0.43); and the sixth visit OR=0.27 (95% CI: 0.19 – 0.39). Intervention-time effect: we found significantly higher adherence in the intervention group at visit four OR=1.59 (95% CI: 1.00 – 2.54); visit five OR=1.67 (95% CI: 1.11 – 2.50); and visit six, OR=2.06 (95% CI: 1.41 – 3.00).
Conclusion: Our findings support the theory that economic strengthening interventions improve patient outcomes and should be incorporated in the care packages for ALHIV in resource-limited settings if the 95-95-95 targets are to be realized.

Effect of economic empowerment intervention on ART adherence among adolescents living with HIV

815 ORAL MICROBIOTA IN CHILDREN WHO STARTED ANTIRETROVIRAL TREATMENT AT YOUNG AGES
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Background: Infancy is an important developmental period when the human microbiome is shaped. Given links between young age at antiretroviral treatment (ART) initiation and smaller persisting viral reservoirs, we hypothesized that earlier ART initiation may have led to distinct microbial signatures in the oral cavity detectable in children living with HIV (CLWH).
Methods: Oral swab samples were collected from 477 CLWH and 123 children without HIV at two sites in Johannesburg, South Africa. CLWH had started ART < 2 years of age with 60% starting < 6 months of age. Most were well-controlled on ART at a median of 10 years of age when the swab was collected. Controls were age-matched and recruited from the same communities. Sequencing of the V4 amplicon of the 16S rRNA gene was done using established protocols. DADA2, decontam, and phyloseq were used for sequence inference, contaminant removal, and subsequent analyses. All p-values were adjusted for multiple testing using Benjamini-Hochberg false discovery rate method. Statistical analyses were performed with R.
Results: CLWH had lower alpha diversity than uninfected children (Shannon index p<0.0001). Genus-level abundances of Gemella, Streptococcus, and Gemella were greater and Neisseria and Haemophilus were less abundant.
among CLWH compared to uninfected children. Associations were strongest among boys. There was no evidence of attenuation of associations with earlier ART initiation. In fact, decreased bacterial diversity and differences in taxa abundances in CLWH versus controls were consistent regardless of whether ART was started before or after 6 months of age. Shifts in genus-level taxa abundances relative to uninfected controls were most marked in children on regimens containing lopinavir/ritonavir; with few shifts seen if on regimens containing efavirenz.

Conclusion: A distinct profile of less diverse oral bacterial taxa was observed in school-age CLWH on ART versus uninfected age-matched children suggesting persisting interference of HIV and its treatments on microbiota in the mouth. Any effects of earlier ART initiation were not detectable at this age. Studies of treated adults with HIV have observed similar shifts in taxa abundances. Oral microbiota have been linked to salivary cytokine levels with associations between Granulicatella and IL-8 and Neisseria and IL-6. Declines in Neisseria abundances in oral samples have been associated with more severe outcomes in influenza and COVID-19.

816 IMMUNE ACTIVATION AND NEUROCognition IN UGANDAN ADOLESCENTS LIVING WITH HIV

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Background: Immune activation is associated with neurocognitive problems in virally suppressed adults living with HIV. Less is known about this relationship in adolescents in perinatally acquired HIV (PHIV) residing in low-income countries with high burdens of PHIV. We examined this relationship in Ugandan adolescents with PHIV.

Methods: Eighty-two adolescents in Kampala, Uganda (28 virally suppressed [<400 copies/mL] PHIV and 54 socio-demographically matched HIV-negative controls) completed 12 tablet-based neurocognitive tests. Control based test z-scores and a global/overall z-score were calculated. We measured plasma (soluble CD14 and CD163), monocyte (percentages of monocyte subsets), and T-cell (expression of CD38 and HLA-DR) activation. Spearman correlations examined associations between test performance and immune activation.

Results: Median (IQR) age was 15(13-16) years; 40% were female. Median time on ART was 10(7-11) years for PHIV; 87% had viral load <50 copies/mL. There were no sociodemographic or immune differences between groups.

Compared to controls, global z-scores were lower among the PHIV group ($p = 0.05$), and significantly worse on executive functioning and delayed recall tests ($p < 0.05$). Overall, monocyte activation significantly correlated with worse test performance: sCD163 with worse global z-score ($r = 0.22, p = 0.04$); sCD163 and non-classical monocytes with worse attention, processing speed, and motor speed ($r = 0.2-0.3, p < 0.01$). T-cell activation (% CD4+ and CD8+ T cells expressing CD38 and/or HLA DR) was significantly associated with worse performance on learning, working memory, and executive functioning tests ($r = 0.2-0.4, p < 0.05$). Similar associations were found by study arm, though among controls, T-cell activation and worse delayed recall were also significantly correlated ($r = 0.32, p = 0.02$).

Conclusion: PHIV with virologic suppression on ART showed evidence of worse neurocognition and similar levels of immune activation compared to controls. For the first time, we showed that monocyte and T-cell activation correlated with worse neurocognition in Ugandan adolescents with PHIV, extending findings from prior studies. However, controls also showed similar associations between immune activation and neurocognition. More research is needed to understand this relationship and its mechanisms in adolescents in low-income countries exposed to myriad sources of immune activation, as well as tremendous social and environmental adversity across neurodevelopment.

817 NEUROCOGNITIVE FUNCTION AMONG SCHOOL-AGED CHILDREN AFFECTED BY HIV IN BOTSWANA

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Background: Children living with HIV (HIV+) have higher rates of cognitive dysfunction, particularly in domains of attention, information processing speed, episodic memory, psychomotor, and executive functioning. HIV-exposed uninfected children (HEU) have been shown in some, but not all, studies to suffer from more neurodevelopmental delays than HIV-unexposed uninfected children (HUU). The Penn Computerized Neurocognitive Battery (PCNB) includes 14 tests that have been adapted for school-aged children in Botswana to assess memory, complex cognition, motor/processing speed, and executive function. We hypothesized that differences between HIV+, HEU and HUU children would be measurable using the PCNB.

Methods: We compared PCNB accuracy, speed, and efficiency scores in 628 children aged 7–18 years, ~1/3 of whom were in each of the following groups: a) HIV+, randomly selected from a clinic; b) HUU, randomly selected from public schools; and c) HEU, 18% of whom were randomly selected as part of the public school recruitment and 82% recruited HEU family members of clinic patients. Scores were age-normed using non-linear multiple regression. Multivariate normative comparisons were used to detect clinically relevant impairment (i.e., below the lower bound of the 95% confidence interval for HUU children) in individuals.

Results: All summary scores and domain scores (e.g., memory) were significantly lower for HIV+ children compared to HUU children, with medium-to-large effect sizes. In nine out of 15 comparisons, the means of the HIV+ and non-randomly selected HEU were contained within each other’s confidence intervals (CIs), indicating some level of equivalence. By contrast, there were no comparisons in which the randomly selected HEU CIs included the HIV+ mean (or vice versa). HUU CIs included the randomly selected HEU mean in six of the 15 comparisons of accuracy, speed, and efficiency. (See Figure for efficiency data.) Multivariate normative comparisons flagged 27% of HIV+, 23% of HEU, and 9% of HUU children as clinically impaired. Of the HEU, 49 of 168 (29%) non-randomly selected and 6/36 (17%) randomly selected children were flagged as neurocognitively impaired.

Conclusion: HIV+ children showed worse neurocognitive function than HUU children in Botswana, supporting the validity of the PCNB in this setting. Interestingly, results also raise questions of whether previous findings of worse performance in HEU children were related to biased sampling or unmeasured confounding.

PCNB efficiency scores stratified by HIV status and cohort
818 TRAUMATIC EVENT EXPOSURE AND MENTAL DISORDERS IN YOUTH PERINATALLY AFFECTED BY HIV
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Background: Traumatic event exposure (TEE) is elevated amongst people living with HIV when compared to the general population and precedes negative outcomes such as mental illness, poor medication adherence, and viremia. Few studies have examined the impact of TEE on any of these outcomes in adolescents and young adults perinatally infected with HIV (PHIV) or perinatally exposed, but uninfected (PHUE). This largely urban and ethnically minoritized population bare a high burden of life stressors and mental illness and may be more vulnerable to TEE effects. This longitudinal analysis examines TEE prevalence experienced in childhood/adolescence among youth with PHIV (YPHIV) and PHUE (YPHUE) and assesses associations of cumulative TEE exposure with psychiatric or substance use disorders in young adulthood.

Methods: YPHIV and YPHUE, ages 9-16 years, were recruited in New York City and interviewed every 12-18 months over 5 follow-ups (FUs). Data come from the DISC-IV, a psychiatric interview. Lifetime TEE data come from youth report at enrollment (mean age=12), FU1 (mean=14), and FU2 (mean=17). Past year psychiatric and substance use disorder data come from FUS, when participants were young adults (mean age=23). Logistic regressions tested associations between cumulative counts of childhood/adolescent TEE and young adult psychiatric and substance use diagnoses.

Results: Among participants (N=237, 54% female, 59% African American, 51% Latino), 39% endorsed ≥4 lifetime TEE; and 21% endorsed 0 or 1 during youth study visits. At FUS, 26% had a past-year psychiatric diagnosis, and 28% had a substance use disorder. Experiencing 4 or more lifetime TEEs vs. 0 or 1 TEE was positively associated with both past-year psychiatric and past-year substance use diagnoses in young adulthood. Those who had been in a situation where they thought someone would be seriously hurt or killed had higher odds of both outcomes; those who had been upset by seeing a dead body had higher odds of a substance use disorder. There were no HIV-status group differences.

Conclusion: These findings provide evidence for premature aging in PHIV adolescents and young adults, and reinforce the relationship between the HIV reservoir and immune senescence. In addition, these findings demonstrate for the first time that the HIV reservoir positively correlates with circulating denervation biomarkers, thus adding new tools for minimally invasive monitoring of biological aging in this population over time.

Spearman correlation plot. Colour scale represents Spearman’s correlation coefficient. Blue and red correspond to positive and negative coefficient, respectively

819 MULTIFACETED PREMATURE AGING IN ADOLESCENT/YOUNG ADULT WITH PERINATALLY ACQUIRED HIV
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Background: HIV infection has become a chronic condition, due to antiretroviral treatment. Adolescents and young adults with perinatal acquired HIV (PHIV) are at risk of developing premature senescence and aging-associated illness. The assessment of aging biomarkers and their relation with the HIV reservoir becomes a priority to characterize and monitor these patients.

Methods: 41 PHIV adolescents/young adults (age 16 - 30 years) under antiretroviral therapy and with undetectable viremia for at least 10 years, and 23 aged-matched healthy controls were enrolled in this study. The immune aging profile (activated, senescent, exhausted and regulatory T and B cells) was studied by flow cytometry. Relative telomere length and HIV-DNA in PBMC were measured by real time PCR and ddPCR, respectively. Circulating denervation biomarkers NCAM-1 (Neural Cell Adhesion Molecule-1) and CAF (C-terminal Agrin Fragment), associated with aging and sarcopenia, were assessed by ELISA. Statistical analyses were performed using RStudio software and data were adjusted by age.

Results: Compared to healthy controls, PHIV subjects had significantly higher levels of immune senescence [%CD4ema/CD28-CD57+: 8.14 (5.26-18.18) vs 3.73 (2.16-4.93), p<0.000, % CD8ema/CD28-CD57+: 6.86 (4.00-12.50) vs 3.45 (2.44-6.25), p=0.003, %CD19+CD10-CD27-IgD-: 10.49 (8.36-14.62) vs 9.20 (4.81-11.82), p= 0.050, exhaustion [%CD4+PD-1+: 11.45 (7.81-13.58) vs 6.58 (5.58-9.52), p< 0.000, %CD8+PD-1+: 11.90 (8.20-13.85) vs 6.75 (4.46-7.36), p< 0.000] and denervation biomarkers [NCAM-1: 364.6 (284.6-757.0) vs 282.5 (241.0-374.2) ng/mL, p = 0.006; CAF: 2282.8 (1989.2-2865.8) vs 2119.5 (1662.9-2356.5) pg/mL, p=0.048]. HIV-DNA positively correlated with immune senescent, activated and exhausted T and B cells, and inversely correlated with regulatory T and B cells and telomere length. Notably, HIV-DNA was also significantly correlated with denervation biomarkers (Table).

Conclusion: These findings provide evidence for premature aging in PHIV adolescents and young adults, and reinforce the relationship between the HIV reservoir and immune senescence. Future work could investigate causal outcomes; those who had been upset by seeing a dead body had higher odds of a substance use disorder. Experiencing 4 or more lifetime TEEs vs. 0 or 1 TEE was positively associated with both past-year psychiatric and past-year substance use diagnoses in young adulthood. Those who had been in a situation where they thought someone would be seriously hurt or killed had higher odds of both outcomes; those who had been upset by seeing a dead body had higher odds of a substance use disorder. There were no HIV-status group differences.

Spearman correlation plot. Colour scale represents Spearman’s correlation coefficient. Blue and red correspond to positive and negative coefficient, respectively

820 CHILDREN WITH PERINATAL HIV EXHIBIT HIGH GDF15 LEVELS DESPITE ANTIRETROVIRAL THERAPY
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Background: Growth differentiation factor-15 (GDF15) is induced by myocardial inflammation to facilitate tissue repair. GDF15 levels were linked with cardiovascular disease (CVD) in adults living with HIV and echocardiographic abnormalities in children with perinatal HIV (CPHIV) >5 years old, but there is limited data in younger CPHIV. We investigated GDF15 in CPHIV ages 2 months to 20 years and their correlations with clinical parameters, immune activation and intestinal damage.

Methods: We quantified GDF15 plasma levels in 232 Kenyan children who were HIV unexposed (HU) and CPHIV and treatment naive (ART-) or virally suppressed on treatment (ART+) aged 0-5 years (“0-5y” n=30 ART-, 30 ART+, 36 HU) and 5-20 years (“5-20y” n=43 ART-, 47 ART+, 46 HU). We assessed correlations between GDF15 and HIV viral load, CD4%, CD4:CD8, monocyte (sCD14, sCD163), T cell (CD38+HLA-DR+ CD4 and CD8 T cells) and systemic (CRP) activation markers and gut mucosal damage (intestinal fatty acid binding protein, IFABP). Plasma biomarkers were measured by ELISA (R&D Systems) and T cell activation by flow cytometry. Kruskall-Wallis test and Spearman’s correlation were performed with GraphPad Prism.

Results: Compared to HU, ART- had higher GDF15 levels in 0-5y (p=0.001) and 5-20y (p=0.0002). In both age groups ART+ had higher GDF15 compared to HU (p<0.0001). 5-20y ART+ had higher GDF15 levels than ART- (p=0.001). There was no correlation between GDF15 and age in HU or CPHIV. GDF15 in 0-5y CPHIV correlated with CD4% (p=0.04, r=-0.26), CD4:CD8 ratios (p=0.03, r=-0.31), IFABP (p=0.004, r=-0.37), sCD14 (p=0.004, r=-0.36), CRP (p=0.03, r=0.28) and CD38+H+LA-DR+ CD4 T cells (p=0.005, r=-0.39). In 5-20y CPHIV, GDF15 correlated with viral load (p=0.04, r=-0.21) and sCD14 (p=0.01, r=-0.27). GDF15 correlated directly with age at ART initiation (p=0.02, r=0.41) and inversely with ART duration (p=0.03, r=-0.39) in ART+ 0-5y but not 5-20y.

Conclusion: CPHIV have elevated GDF15 levels compared with HU starting in early childhood regardless of age and treatment. In younger CPHIV, high GDF15 levels linked with advancing HIV and global inflammation. In older CPHIV, GDF15 levels were independent of inflammatory markers but higher in ART+ compared to ART-. Our data suggest that myocardial inflammation may begin early in perinatal HIV and continue despite ART in older children. Starting ART at younger ages may mitigate cardiovascular comorbidities in CPHIV but needs further study with clinical CVD measures.
**821** 48 MONTHS ANTHROPOMETRIC EVOLUTION OF ADOLESCENTS SWITCHING TO DOLUTEGRAVIR IN SPAIN

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**Background:** Dolutegravir (DTG) is now recommended as a first-line treatment for people living with HIV. However, clinical trials among adults have risen concerns regarding DTG-associated weight gain, and real-life data addressing weight gain in adolescents are still scarce. Anthropometric changes are hard to evaluate and a concern, especially during adolescence, a period characterized by changes in body composition. We compare the evolution of a cohort of children and adolescents who switched to DTG-based regimens vs. those who maintained their previous regimens.

**Methods:** Retrospective study within the Spanish Cohort of Pediatric HIV. Participants < 18 years of age switching to a DTG-containing regimen before December 2020 and with at least 6 months of follow-up data were included. Patients under follow-up in the same cohort and not receiving DTG or bictegravir were included for comparison. The WHO growth charts were used to estimate zBMI. Linear mixed models were built to model the treatment effects on zBMI over 48 months.

**Results:** We included a total of 275 patients (135 switching to DTG), 49% female. The median age was 13.6 ± 2.9 years (48% Caucasian, 28% Black, 9% Latino). DTG regimens mostly included abacavir plus lamivudine (75%). The third drug in the control group included protease inhibitors (45%) efavirenz (32%), or elvitegravir/raltegravir (21%). At baseline, the prevalence of overweight and obesity were 20% and 6.5%, respectively. Both groups were comparable regarding age, sex, ethnicity and baseline zBMI as covariates, subjects switching to DTG experienced a greater adjusted zBMI increase than those remaining on their previous treatment (p=0.027) (Figure 1), with no significant changes in subgroups by changes in body composition. We compare the evolution of a cohort of children and adolescents who switched to DTG-based regimens vs. those who maintained their previous regimens.

**Conclusion:** Our observational study shows an effect of DTG switch on zBMI, already significant at 12 months and maintained up to 48 months of follow-up, mainly affecting black adolescents. Despite the risk of residual confounders, our results are consistent with previous experiences in adults suggesting a higher risk of weight gain in the black population. Larger studies are needed in order to analyze the long-term effects of DTG among children and adolescents, ideally including more precise assessments of body composition.

Figure 1. Anthropometric evolution according to treatment (all patients)

**822** METABOLIC HEALTH AND BONE DENSITY IN YOUTH LIVING WITH PERINATAL HIV

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**Background:** Adverse metabolic profiles in adults living with HIV are associated with traditional risk factors, HIV virämia and antiretroviral therapy (ART) with a paucity of data for youth living with perinatally acquired HIV (YPaHIV). As body mass index (BMI) impacts metabolic health and bone mineral density (BMD), we explored the relationship between markers of metabolic health, body fat distribution, hepatic steatosis and BMD in YPaHIV.

**Methods:** A longitudinal observational study examined bone and metabolic health in YPaHIV aged 15-24 years by total body dual-energy X-ray absorptiometry (DXA), liver fibroscan and fasting metabolic biochemistry. Associations of metabolic markers with DXA and fibroscan outcomes and by tenefinov alafenamide (TAF) use were made using two-sample t-tests and correlations investigated by Pearson’s coefficient. Metabolic risk factors for lumbar spine (LS) BMD were assessed by linear regression.

**Results:** 85 participants; 49 (58%) male, 80 (94%) black/black African, median age 22 years (IQR 19-24), and CO4 count 645 (IQR 373-917) cells/µl, were followed for 26 months (IQR 25-28). Mean (SD) systolic blood pressure was 120 (12), fasting lipids (triglycerides 0.7 [0.9], HDL 1.4 [0.4]) and glucose 4.5 (0.5). Baseline BMI was 25.7 (5.4) increasing at 26 months by TAF (n=44) versus non-TAF (n=41) by 0.6 (2.7), 1.4 (2.6), (p=0.02) with weight gain of 2.2 (7.8) and 3.7 (6.3), (p=0.34) respectively. Correlation (r=0.05) with BMI included BMI 0.23 (0.03), fasting lipids (total cholesterol -0.33 [0.002], triglycerides -0.23 [0.03], LDL -0.31 [0.004]), total body -0.26 (0.02) and gynoid -0.26 (0.02) fat. Correlation with controlled attenuation parameter (CAP) score included; BMI 0.27 (0.01), waist circumference 0.29 (0.007) and fasting glucose 0.3 (0.02) but not lipid or body fat parameters. Hepatic transaminases correlated with E score (ALT 0.56 [< 0.0001], AST 0.42 [0.0001]) but not CAP. Factors associated with BMD were shown in table 1. Metabolic factors according to TAF vs versus non-TAF differed only for total cholesterol; mean (SD) 4.4 (0.9) versus 4.0 (1.0), (p=0.03) with no differences seen for triglycerides, BMI, weight, waist circumference and body fat distribution.

**Conclusion:** Metabolic factors including obesity, gynoid fat distribution and abnormal lipid profiles were associated with adverse bone health in this youth cohort living with PaHIV. Effective interventions targeting traditional risk factors are required. Linear regression of factors associated with lumbar spine bone mineral density

**823** THE BONDY STUDY: BONE DENSITY IN YOUTH LIVING WITH PERINATALLY ACQUIRED HIV

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**Background:** Youth living with perinatally acquired HIV (YPaHIV) have multiple risk factors for bone mineral density (BMD); early years malnutrition, impaired growth, delayed puberty, and the cumulative effects of antiretroviral therapy (ART) including tenofinav disoproxil fumarate (TDF). Tenovifin alafenamide (TAF) is recommended as an alternative to TDF in those < 25 years due to an increased safety profile. We present longitudinal data on BMD accrual in YPaHIV on TAF vs non-TAF, non-TDF ART.

**Methods:** A longitudinal, observational study (London U.K.) evaluated bone health in YPaHIV aged 15-19 years (n=50), 20-24 (n=50) and 25+ (n=30) by dual-energy X-ray absorptiometry, bone biochemistry and turnover markers.
vitamin D and parathyroid hormone (PTH) with 2-year follow-up evaluation in 15-19 years (n=42) and 20-24 (n=43). Abnormal BMD was classified as osteopenia (Z/T score < -1) or osteoporosis (Z/T score < -2). T-tests compared results between TAF and non-TAF ART. Linear regression identified factors associated with low BMD and 2-year BMD accrual. Results: At baseline 74/130(57%) were female, 106(82%) black, median age 21[Q1R 18-24] years and CD4 count 707/1845 - 925 cells/μL. 2-4 and/or femur BMD matched for age, sex and ethnicity was abnormal in 13(26%) 15-19 years. 25(56%) 20-24 and 196(93%) 25+. Vitamin D was < 50nmol/l in 4284(44%), 3672% and 2170% with high PTH (>7.2 pmol/l) in 1224%, 2448% and 1137%. 85 followed up at a median of 26[Q1 25-28] months; 4452% on TAF. Changes in BMD and bone mineral content (BMC) from baseline were shown (Table). There was no difference in BMC delta change with TAF, except for lumbar spine (LS BMDC in 15-19 years (B=0.07; p=0.54) TAF vs 0.18; p=0.48 non-TAF, B=0.08; p=0.39). Predictive factors of LS BMD change were baseline DXA (B=0.16 per 1 higher, 95%CI -0.32, 0.01; p=0.04). No association was seen with TAF use, duration, age, sex, BMI, mobility, bone markers (NTX, PINP), PTH, vitamin D or alkaline phosphatase (ALP). LS BMD change was weakly associated with TAF duration (B=0.04 per month longer; 95% CI -0.01, 0.10; p=0.09) and ALP (B=0.17 per 10 higher; 95% CI -0.01, 0.15; p=0.04). In those 20-24 years, greater BMD hip change was associated with PTH (B=0.01 per 1 higher, 0.02; p=0.00; p=0.01).

Conclusion: Reassuringly longitudinal BMD accrual was in keeping with an age, sex and ethnicity-matched population. We found limited evidence of a difference in BMD accrual favouring those receiving TAF-ART compared to non-TAF, non-TDF containing regimens. Table. Mean and Standard deviation bone mineral density and bone mineral content of YPaHIV at baseline and 26 months, stratified by age group.

Results: Of 1204 enrolled, 127(11%) were CLWH and of similar age (median[IQR] 19.8[16-21] years for YWHIV who newly initiated ART and 19.7[16-21] years for prevalent ART users, respectively. Time to incident TB was 21.3 and 295.1 days for YWHIV who newly initiated ART and prevalent ART users, respectively. There were no deaths in the follow-up period, 47 YWHIV had a new TB diagnosis; TB incidence was 7.0/1,000 person years (PY) among YWHIV newly initiating ART vs. 3.1/1,000 PY those already on ART. There was no difference in TB incidence by HIV status. Among YWHIV newly on ART over the age of 18, those who had HIV treatment support had a lower risk of TB disease (HR 0.30 [95% CI 0.10-0.97] p=0.04). Among YWHIV newly on ART over the age of 18, those who had HIV treatment support had a lower risk of TB disease (HR 0.30 [95% CI 0.10-0.97] p=0.04). YWHIV with prevalent ART use had lower risk of TB disease if they received cART (HR 0.13 [95% CI 0.05-0.31] p=0.01) compared to those who did not. In multivariable models, TB incidence was higher in CLWH (aHR [95%CI] 2.6 [1.5, 4.3]) and those with CD4 < 350 cells/μL (aHR 3.6 [1.8, 7.1]) compared to those with CD4 ≥ 350 cells/μL.

Conclusion: In children with non-severe TB, deaths occurred early during ART with higher mortality in CLWH. Young age, malnutrition and anemia independently predicted mortality in both CLWH and HIV-uninfected children. Suboptimal viral suppression was common among CLWH, with worst suppression on LPV/r. There was no evidence that CLWH needed longer ART than HIV-uninfected children, and therefore can receive 4 months ART for non-severe TB.

825 TUBERCULOSIS INCIDENCE AMONG ADOLESCENTS AND YOUNG ADULTS WITH HIV IN KENYA

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Background: There is a lack of data regarding the risk of TB among adolescents from clinical trials among adolescents from 1/1/2016 - 1/18/2018 to estimate incidence rates (IR) and evaluate individual characteristics associated with TB disease from date of antiretroviral therapy (ART) initiation (among new ART users) or from the start of the date of the study. Among YWHIV newly initiating ART (1/1/2016 - 1/18/2016) ART users, univariate Cox proportional hazard regression was conducted to estimate hazard ratios (HR). Separate analyses were conducted for adolescents receiving new ART (N=183) and those already on ART (N=137). Among YWHIV newly initiating ART over the age of 18, those who had HIV treatment support had a lower risk of TB disease (HR 0.30 [95% CI 0.10-0.97] p=0.04). YWHIV with prevalent ART use had lower risk of TB disease if they received cART (HR 0.13 [95% CI 0.05-0.31] p=0.01) compared to those who did not. In multivariable models, TB incidence was higher in CLWH (aHR [95%CI] 2.6 [1.5, 4.3]) and those with CD4 < 350 cells/μL (aHR 3.6 [1.8, 7.1]) compared to those with CD4 ≥ 350 cells/μL.

Conclusion: YWHIV with a high incidence of TB, particularly among those newly initiating ART. Therapeutic (cART) and interpersonal (partner testing and treatment support) factors influenced TB incidence in this population. It is important to optimize TB prevention and case finding in this vulnerable population.

Figure 1. Kaplan Meier survival estimates for incident TB among (A) YWHIV with prevalent ART and (B) YWHIV newly initiated ART.
PK & SAFETY OF ABC/3TC DISPERSEABLE TABLETS & LPV/r GRANULES IN NEONATES: PETITE STUDY
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PETITE Study Team
1Stellenbosch University, Cape Town, South Africa, 2Chiang Mai University, Chiang Mai, Thailand, 3University of California San Diego, San Diego, CA, USA
Background: No oral solid fixed-dose combination (FDC) antiretroviral dispensable formulations are available for HIV prevention or treatment from birth. Neonates could benefit from existing antiretroviral solid dispersible formulations. We evaluated the pharmacokinetics (PK) and safety of pediatric ABC/3TC dispersible tablets and LPV/r (2-in-1) granules in term neonates exposed to HIV, birth weights ≥2.0 to ≤4.0 kg.
Methods: The ‘PETITE’ study was a phase I/I, open-label, single-arm, clinical trial in South Africa evaluating oral ABC/3TC (120/60 mg) dispersible tablets and LPV/r (40/10mg) granules. Neonates stratified by birth weight received 30/15 mg of ABC/3TC daily (¼ dispersible tablet) and 80/20 mg of LPV/r twice daily (2 sachets) from birth through 28 days. Two intensive PK sampling visits were performed: one at <14 days of life and after ≥72 hours of treatment (PK1), and another 10-14 days later (PK2). Safety visits were performed 1-2 week(s) after each PK visit. Electrocardiograms (ECGs) were performed at baseline and during follow-up.
Results: Sixteen neonates (15 breastfed) started ABC/3TC + LPV/r within 3 days of life, median (range) birth weight was 3.1 (2.2-3.8) kg. PK1 and PK2 visits were performed between 6-14 and 19-24 days of life, respectively; 2 neonates did not complete PK2. ABC, 3TC doses were 9 (7-13), 4 (3-7) mg/kg once daily, LPV dose was 377 (307-480) mg/m2 twice daily. Geometric mean (GM, 90% CI) AUC0-24 of ABC and 3TC were higher at PK1 versus PK2 - Table 1. ABC exposures at PK1 crossed the upper target range but rapidly decreased to within range by 2-3 weeks of life. 3TC and LPV exposures were within the reported ranges for young infants. Geometric mean ABC, 3TC, and LPV AUC0-24 were 1.79 vs 1.21, 63.4 vs 43.4 mg.hr/L, respectively. No Grade 2 or higher adverse events were related to study drug. All ECGs were normal except for a single Grade 1 prolonged QTcF interval which spontaneously resolved.
Conclusion: Initiating pediatric solid FDCs of once daily ABC/3TC dispersible tablets in combination with twice daily LPV/r granules from birth was safe and well tolerated. ABC and 3TC drug exposures were initially high but rapidly decreased. LPV exposures were lower than in adults but comparable to young infants receiving LPV/r liquid. This study supports giving solid dispersible FDCs from birth for HIV prevention and treatment in neonates.

Table 1: Geometric mean (90% CI) of ABC, 3TC and LPV PK parameters in neonates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC (μg/L)</td>
<td>6.83 (5.88-9.00)</td>
<td>10.1 (8.01, 13.4)</td>
</tr>
<tr>
<td>3TC (μg/L)</td>
<td>4.70 (4.82-5.60)</td>
<td>6.10 (5.20-7.10)</td>
</tr>
<tr>
<td>LPV (μg/L)</td>
<td>11.7 (11.6-11.8)</td>
<td>14.2 (14.0-14.4)</td>
</tr>
</tbody>
</table>

287 TWICE DAILY 50 MG Dolutegravir in Tuberculosis-HIV Coinfected Children 20-35 kg
Anushka Naidoo1, Kajieleum Naidoo2, Gabriella Croieghout3, Marothi Letsoalo4, Mounier Almett5, Roeland E. Wansma6, Paolo Denti6, Lubbe Wiesner6, Kelly E. Dooley1, Moheddan Archariy1
1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2University of KwaZulu-Natal, Durban South Africa, 3University of Cape Town, Cape Town, South Africa, 4Vanderbilt University Medical Center, Nashville, TN, USA, 5Helson R Mandela School of Medicine, Durban, South Africa
Background: Indication for dolutegravir, dosed at 50 mg daily, was used to summarise their exposures before the summary. For children receiving 50mg DTG BD with Rif, the AUCE12/24 was calculated by dividing the AUCE12 by the concentration (C12) at a 24-hour mark, was predicted at 12 hours for two children. While receiving 50 mg DTG OD without Rif, C12 was calculated at 24 hours and AUCE12/24 was calculated by extrapolating up to 24 hours, and C12 was calculated at 24 hours.
Caregivers of children with HIV in Botswana Prefer Monthly IV bNAbs to Daily Oral ART
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1Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 2Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 3Harvard Medical School, Boston, MA, USA, 4Harvard TH Chan School of Public Health, Boston, MA, USA, 5Massachusetts General Hospital, Boston, MA, USA, 6Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 7Harvard Medical School, Cambridge, MA, USA
Background: Monthly intravenous infusion of broadly neutralizing monoclonal antibodies (bNAbs) may be an attractive alternative to daily oral antiretroviral treatment (ART) for children. Acceptability by caregivers remains unknown.
Methods: We evaluated monthly infusion of dual bNAbs (VR0105S and 10-1074) as a treatment alternative to ART among children participating in the Tatelo Study in Botswana. Eligible children aged 2-5 years received 8-32 weeks of bNAbs overlapping with ART, and up to 24 weeks of bNAbs alone, at monthly intravenous infusion visits. Using closed-ended questionnaires, we evaluated the acceptability of each treatment strategy among caregivers prior to first bNAb administration visit (pre-intervention) and after completion of final bNAb administration visit (post-intervention).
Results: Twenty-five children completed all study phases, and acceptability data were available from 24 caregivers at both time points (1 primary caregiver was unavailable at pre-intervention visit). Responses were provided by the child’s mother at both visits (60%), an extended family member at both visits (28%), or a combination of mother and extended family member (12%). Caregiver acceptance of bNAbs was extremely high both pre- and post-intervention, with 21/24 (87.5%) preferring bNAbs to ART pre-intervention, and 21/25 (84%) preferring bNAbs post-intervention (9/21 cared for a child who remained virally suppressed, 12/21 for a child with a viral rebound on bNAbs) (Fig 1). No caregivers preferred ART pre-intervention; however, 2/25 preferred it post-intervention (1 cared for a child who remained virally suppressed, 1 for a child with viral rebound on bNAbs). Pre-intervention, 3 (13%) caregivers analyses were conducted using the nacp package in R. The area under the concentration-time curve (AUC) was calculated using the linear-up log-down trapezoidal method. Extrapolation was done from the last measurement to 12 or 24h, using the elimination rate constant. Participants underwent frequent clinical and safety visits. Viral load was measured at weeks 8, 12, 24 and 48.
Results: We enrolled 13 children between August 2021 and September 2022 and report the preliminary analysis of all data collected up to November 2022; Median (IQR) age 10 (9-11) range 5-13 years; 54% males; 100% black race. The median (IQR) viral load and CD4 at baseline were 2.5 (1.6-5.0) log10 copies/mL and 108.5 (76.5-352.2) cells/mm3. Viral load was undetectable in all children completing the week 12 and 24 visits. There were two grade 3 adverse events and no SAEs. Data from 12 children were included in the preliminary PK analysis, contributing 12 profiles on twice daily dolutegravir and rifampicin and 2 profiles on once daily dolutegravir (total of 114 dolutegravir plasma concentration values). Median trough concentration (C_{trough}) was 1.60 and 1.49 mg/L, while AUCE12/24 was 33.55 and 36.67 h·mg/L for participants on bNAb dolutegravir with rifampicin and OD dolutegravir, respectively. All C_{trough} were > 0.3 mg/L. Results are summarized in Table 1.
Conclusion: Preliminary data from children 20-35kg receiving twice-daily dolutegravir during TB treatment suggest this dosing is well-tolerated and achieves similar PK values to daily treatment for HIV-alone. VL suppression data are, similarly, promising.

Table 1. Dolutegravir (DTG) pharmacokinetic parameters and their unit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/L)</td>
<td></td>
<td>65.4 (44.3, 96.5)</td>
</tr>
<tr>
<td>Cmin (µg/L)</td>
<td></td>
<td>2.34 (1.45, 3.88)</td>
</tr>
<tr>
<td>AUCE12/24 (µg·h/L)</td>
<td></td>
<td>166 (136, 216)</td>
</tr>
<tr>
<td>AUCE12/24 (µg·h/L)</td>
<td></td>
<td>130 (109, 161)</td>
</tr>
</tbody>
</table>

288 CAREGIVERS OF CHILDREN WITH HIV IN BOTSWANA PREFER MONTHLY IV bNAbs TO DAILY ORAL ART
had no preference between bNABs or ART, and 2 (8%) had no preference post-intervention. Pre-intervention, the most common reasons for preferring bNABs over ART were "if infusions were at the same time as other medications vs. ART" (n=10) and "if infusions continued to be once monthly compared to daily ART" (n=9). Post-intervention, no dominant reason for preferring bNABs over ART emerged from caregivers.

**Conclusion:** Monthly intravenous bNAB infusions were highly acceptable to caregivers of children with HIV in Botswana and preferred over standard ART by most. Our findings suggest that caregiver acceptability is an unlikely barrier to bNAB uptake and eventual programmatic use for children living with HIV.

Figure 1. Pre- and post-intervention preferences by caregivers for monthly bNAB infusions vs. daily ART (if medical benefit were equal)

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**830 BIC/FTC/TAF IN FRENCH CHILDREN: FREQUENT VIRAL FAILURE BUT RARE ACQUIRED RESISTANCE**

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**Necker Hospital, Paris, France**

**Background:** Bictegravir-50mg/Emtricitabine-200mg/Tenofovir alafenamide-25mg (BIC/FTC/TAF) is now approved for use in HIV+ children weighing ≥25 kg. However, published data about its efficacy in the paediatric population are limited to one clinical trial including only subjects with suppressed viremia at baseline, good adherence to treatment and short-term follow-up. This “real-life” study aimed to provide long-term data about the risk of viral failure (VF) and acquired genotypic resistance in children and adolescents receiving BIC/FTC/TAF and followed at Necker Hospital (Paris, France).

**Methods:** This retrospective monocentric study included 60 paediatric patients aged <18 years who received BIC/FTC/TAF in 2019-2022. VF was defined as not achieving a viral load (VL) <50 copies/ml within three months of BIC/FTC/TAF initiation or as experiencing viral rebound ≥50 copies/ml.

**Results:** Most of the individuals were antiretroviral experience (93.3%), were previously exposed to INSTI (mainly dolutegravir, 85%), and displayed viral suppression at baseline (63.4%). Their median age was 11.1 years (IQR: 8.9-14.4). In most ARV experienced subjects, B/C/T/F/ART introduction reduced treatment burden compared to previous regimen, which contained multiple pills (61%) or syrups (30%), twice-daily dosages (43%) and/or larger pills size (4%). Genotypic susceptibility score of B/C/T/F/ART was ≥2.5 in all cases. INSTI-associated resistance associated mutations (RMs) were previously isolated in 8 patients: E157F (n=3), L174I (n=5). Median follow-up was 29 months (IQR: 19-35). VF occurred in 23 persons (38.3%), more frequently in case of baseline VL ≥50 copies/ml (57.1% versus 28.2%, p<0.05). Compared to children with sustained viral suppression, those with VF had higher median baseline VL (4.5 versus 2.7 log10 copies/ml, p=0.02) and longer median duration of follow-up (33 versus 27 months, p=0.03). No emergence of RMs was observed in patients with VF, despite a long duration of viremia while on treatment (median 7.5 months [IQR: 6-23]). With reinforced measures to improve adherence, undetectable VL was obtained at the last visit in 81.7% of patients without requiring ART change.

None patient stopped B/C/T/F/ART for drug-related side effect.

**Conclusion:** Because of its good tolerability, its high genetic barrier to resistance and small pill burden, B/C/T/F/ART could be especially useful in the paediatric population, in which the risk of poor treatment adherence is high.

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**831 ADEQUATE DTG EXPOSURE IN INFANTS ON RIFAMPICIN TREATMENT RECEIVING TWICE-DAILY DTG**

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**EMPIRICAL clinical trial group**

**1University of Zimbabwe, Harare, Zimbabwe, 2University of the Witwatersrand, Johannesburg, South Africa, 3New England Medical Center, Boston, MA, USA, 4Mahidol University, Bangkok, Thailand, 5Khan Kain University, Khan Kain, Thailand, 6Gilead Sciences, Inc, Dublin, Ireland, 7Gilead Sciences, Inc., Foster City, CA, USA**

**Background:** Dolutegravir (DTG) is recommended by WHO as first-line treatment option for children living with HIV. Rilpivirine (RIP) interacts...
substantially with DTG by increasing DTG metabolism through induction of UGT1A1 and CYP3A4, thereby reducing its exposure resulting in potential treatment failure. Adapting the DTG dosing interval from once-daily (OD) to twice-daily (BID) was safe and effective in both adults and children. However, no pharmacokinetic (PK) data was available for children weighing < 14 kg. The outcome of the RIF-DTG interaction may be different in infants as maturation of metabolic enzyme activity may not have been completed. PK data in infants receiving DTG and concomitant RIF is warranted and was identified as a knowledge gap by WHO. We evaluated plasma DTG concentrations in infants living with HIV receiving DTG BID with RIF-based TB-treatment.

Methods: This is a 2-arm PK sub-study of the EMPERIAL randomized controlled trial (NCT03915366) for severe pneumonia in infants living with HIV. Eligible infants aged 1-11 months, weighing ≥ 3 kg, receiving DTG OD (control) or DTG BID with RIF-based TB-treatment, were recruited in Mozambique, Uganda, Zambia, and Zimbabwe. Infants received DTG following WHO weight-band dosing. Six blood samples were taken over 12 (BID) or 24 (OD) hours 30-90 days after start of RIF and at least 14 days after initiation of DTG. Relevant PK parameters and the proportion of infants with DTG Ctrough below the PK target (0.32 mg/L) were summarised per treatment arm. This project is part of the EU FP7A 2017MC-2 programme supported by the European Union.

Results: Of 30 enrolled infants, 27 had evaluable PK curves (Figure) of which 21 received concomitant RIF. The median (IQR) age was 7.1 (6.1 – 9.9) months, weight 7.1 (6.1 – 9.9) months, and 11/27 were female. DTG Cmax, AUC0-24h, and Ctrough, GM (%CV) were 1.05 (82) mg/L, 49.7 (70) h*mg/L, and 3.36 (65) mg/L for children on RIF, and 1.11 (46) mg/L, 54.3 (49) h*mg/L, and 3.86 (38) mg/L for infants in the control arm, respectively. Only 1/21 infants in the RIF arm had DTG Ctrough < 0.32 mg/L vs none out of 6 in the control arm. Apparent oral DTG clearance was about 2-fold higher for infants receiving RIF.

Conclusion: Consistent with data from older children and adults, BID dosing of DTG when administered in infants on RIF resulted in adequate DTG exposure. These PK data support the use of DTG BID for infants living with HIV receiving RIF.

832 MODEL-INFORMED DOLUTEGRAVIR DOSE SELECTION IN PEDS WITH 1ST GENERATION INSTI-R

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1GlaxoSmithKline, Collegeville, PA, USA, 2ViiV Healthcare, Durham, NC, USA, 3GlaxoSmithKline, Brentford, United Kingdom, 4Clinton Health Access Initiative, Boston, MA, USA, 5Baylor College of Medicine, Houston, TX, USA, 6WV Healthcare, Brentford, United Kingdom, 1University of California San Francisco, San Francisco, CA, USA, 7University of California Berkeley, Berkeley, CA, USA

Background: Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) approved for once-daily dosing in INSTI-naive adults and children living with HIV. For adults with INSTI resistance (INSTI-r) based on certain substitutions or clinical suspicion, current recommendations are to double the standard 50 mg dose of DTG giving it twice daily (BID). However, dosing recommendations for children with INSTI-r have not been assessed. Our objective was to generate model-based population pharmacokinetic (PopPK) data to inform DTG BID dosing in 1st generation INSTI-r (raltegravir or elvitegravir) children (age ≥ 4 weeks and weight ≥ 3 kg), extrapolating efficacy and safety from adults to pediatric population based on drug exposures, in accordance with FDA andEMA guidance.

Methods: A PopPK model was developed using data from IMPAACT P1093 (NCT01302847) and PENTA ODYSSEY (NCT02259127) to predict exposures from BID dosing of DTG in pediatric patients. Clinical trial simulations were performed to evaluate predicted drug levels with different dosing strategies. Exposure metrics (AUC0-24h, Cmax, and Ctrough) were calculated for each dose within each weight band. The target PK exposures (Cmax geometric mean >1.97 µg/mL & AUC0-24h >32.2 µg*h/mL) for pediatrics were selected based on this adult data. DTG Cmax exposures were also evaluated for safety, with reference to existing adult and pediatric data.

Results: The BID dosing using the dose approved for once-daily dosing in INSTI-naive children exceeded Cmax exposures in several weight-bands compared to existing adult and pediatric data. Among the several dosing strategies evaluated, the proposed BID dosing (Table 1) yielded predicted exposures within each weight band that were comparable to the pre-defined adult exposures. These proposed doses are expected to provide similar efficacy as observed in adults with 50 mg BID dosing.

Conclusion: The proposed BID dosing for children is predicted to achieve similar drug exposures to those in adults. While clinical PK data would form an ideal basis for dosing, recruitment of children with 1st generation INSTI-r is exceedingly challenging. These modeled data inform DTG dose selection for pediatric patients with 1st generation INSTI-r (raltegravir and elvitegravir). It is important to note that this dosing differs from guidance in adults (double standard dosing) and that BID dosing of DTG is not expected to be effective against viral resistance to DTG or bicitigravir.

Table 1. Dolutegravir Model-Based BID Dosing Regimens in 1st Generation INSTI-r Pediatric Subjects

<table>
<thead>
<tr>
<th>Weight Band (kg)</th>
<th>Discontinuous Tablet BID Dose</th>
<th>Cmax (µg/mL)</th>
<th>AUC0-24h-12h (µg*h/mL)</th>
<th>C12h (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3 to &lt;6</td>
<td>5 mg</td>
<td>1.3 (1.0 - 1.7)</td>
<td>(1.0 - 1.7)</td>
<td>5.0 (4.0 - 6.0)</td>
</tr>
<tr>
<td>≥6 to &lt;10</td>
<td>10 mg</td>
<td>1.9 (1.6 - 2.3)</td>
<td>(1.6 - 2.3)</td>
<td>9.5 (8.0 - 11.5)</td>
</tr>
<tr>
<td>≥10 to &lt;14</td>
<td>15 mg</td>
<td>2.6 (2.2 - 3.1)</td>
<td>(2.2 - 3.1)</td>
<td>14.0 (12.0 - 16.0)</td>
</tr>
<tr>
<td>≥14 to &lt;20</td>
<td>20 mg</td>
<td>3.3 (2.8 - 3.9)</td>
<td>(2.8 - 3.9)</td>
<td>18.5 (16.5 - 21.5)</td>
</tr>
<tr>
<td>≥20 to &lt;30</td>
<td>30 mg</td>
<td>4.1 (3.6 - 4.7)</td>
<td>(3.6 - 4.7)</td>
<td>25.0 (22.0 - 28.0)</td>
</tr>
<tr>
<td>≥30 to &lt;40</td>
<td>40 mg</td>
<td>5.0 (4.5 - 5.6)</td>
<td>(4.5 - 5.6)</td>
<td>33.5 (31.0 - 36.0)</td>
</tr>
</tbody>
</table>

Impact of Malnutrition on Dolutegravir Exposure and Alternative Dosing

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Background: Despite improvements in access to antiretroviral therapy (ART) worldwide, HIV/AIDS remains a major cause of child mortality, with 120,000 children dying of HIV-related illnesses in 2020. Among children receiving ART, malnutrition is associated with higher mortality. However, current dosing guidelines for many ARTs do not account for age or nutritional status. Thus children of healthy weight are often given the same dose as malnourished children of the same weight, irrespective of disparities in metabolic capacity due to their age differences. Low ART exposure may contribute to mortality in malnourished children with HIV. In order to assess the potential impact of nutritional status on ART exposures, we simulated pharmacokinetics (PK) for dolutegravir (DTG) in a real-world global population of children under 5 years of age.

Methods: Anthropometric data from the 30 countries with the largest populations of children living with HIV were acquired from demographic health surveys. A population PK model was used to simulate DTG exposures. WHO guideline dosing was compared to: a) a modified algorithm which used expected weight for age (as opposed to actual weight) for malnourished children, and b) model-based dosing derived from individual PK parameters. Both alternative methods require only weight, age, and sex as inputs. Target geometric mean (GM) C24 was 0.95 µg/mL, with an acceptable individual minimum C24 of 0.500 µg/mL. Simulations were performed with NONMEM 7.4.3.

Results: In the anthropometric dataset, 389216 children aged 0-5 years were included, and 26% of these were underweight, defined as having a weight-for-age z-score (WAZ) below -2. Considering the estimated pediatric HIV incidence in each country, we predict that as many as 13,941 and 17,101 children under 5 could be brought above the individual minimum target by changing from guideline dosing to expected-weight and model-based dosing, respectively. Among underweight children, predicted GM C24 was 0.78, 1.03, and 1.29 µg/mL with guideline, expected-weight, and model-based dosing, respectively.

Conclusion: Current dosing guidelines, which are weight-based, may need to account for age and nutritional status to prevent under-exposure of first-line

833 IMPACT OF MALNUTRITION ON DOLUTEGRAVIR EXPOSURE AND ALTERNATIVE DOSING
ART drugs such as dolutegravir and improve outcomes in young, malnourished children with HIV.

Simulated DTG target attainment

834 NITALMATREVIR-RITONAVIR TREATMENT IN CHILDREN WITH SARS-CoV-2 INFECTION

Stefania Bernardi1, Costanza Tripiciano1, Stefania Mercadante1, Anna Markovich1, Giulia Lorenzetti1, Francesca Calo Carducci1, Lorenza Romani1, Laura Cursi1, Emma Concetta Mannu’, Federica Galaverna’, Leonardo Vallesi’, Massimiliano Raponi1, Emanuele Nicastri1, Carlo Federico Perno1, Paolo Rossi1

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Background: Children seem to experience a less severe form of COVID-19 disease than adults, nevertheless, cases of severe infection have been described in a small proportion of patients, requiring hospitalization in 5-10% of cases. Among COVID-19 deaths 0.4% occurred in children and adolescents under 20 years of age. Most hospitalized children with acute COVID-19 had underlying conditions. Moreover, some children with previous COVID-19 infection, may later develop Multisystem Inflammatory Syndrome in Children (MIS-C), a rare but serious condition associated with COVID-19. These data suggest that a specific therapy is necessary in high-risk pediatric population, in order to prevent severe COVID-19, especially in children with underlying conditions. Antiviral paediatric data are currently very few.

Methods: We conducted a retrospective study on patients < 18 years of age who received Paxlovid (nirmatrelvir-ritonavir) for the treatment of mild-to-moderate COVID-19 at Bambino Gesù Children’s Hospital from April 2022 to September 2022. Patients at high risk of progression to severe COVID-19 who had no need of supplemental oxygen received Paxlovid according to AIFA’s indications for adults with the Informed Consent of relatives.

Results: 40 patients aged 1-18 years with confirmed SARS-CoV-2 infection were treated with Paxlovid (Tab 1). The average symptom duration was 4.2 days. No patient developed severe COVID-19. All patients were treated within 5 days of symptom onset. Four patients received a longer course treatment (10 days) due to the persistence of symptoms combined with the presence of severe comorbidities. The mean time of viral shedding was 12.7 days, with a patient being persistently positive for 56 days. After Paxlovid initiation, only 5 patients (12.5%) experienced adverse reactions:

Conclusion: Treatment with Paxlovid has proven to be safe. Further pharmacokinetic studies are required species for children < 5 years old.

835 COMORBIDITIES THAT INCREASE RISK FOR SEVERE ACUTE COVID-19 IN PEDIATRIC POPULATION

Alfredo Tagarro1, Irati Gastesi2, Andrea Ramirez Valera3, María Lucia Mesa Rubio4, Pablo Vázquez Hoyos5, Gabriela Friedrich6, Melisa Naranjo Vanegas7, Sonia Restrepo-Gualteros8, Olga Lucia Baquero9, Inés Leez Gordillo10, Cristina Calvo Rey11, Siobhan Crichton12, Marthe Le Prevost13, Carlo Giaquinto14, Cinta Moraleda15

EPICO, EPICO Colombia and ORCHESTRA

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Background: Little is understood about which comorbidities are associated with severe outcomes in children hospitalized with acute COVID-19. Some confusion lies especially for cancer or diabetes.

Methods: Data from 2 multicenter prospective cohort studies of hospitalized children (aged 0-18 years) with confirmed SARS-CoV-2 in Spain and Colombia were combined for this analysis. Data were obtained from 116 hospitals. Outcome was classified as (in decreasing order of severity): death, mechanical ventilation (MV), pediatric intensive care unit (PICU) admission, high flow/CPAP, oxygen therapy with nasal prong (NP) and hospitalization without respiratory support. Risk factors for severity, adjusting for age and gender, were identified using multinominal logistic regression and a backwards selection process.

Results: A total of 1,753 patients were included, 734 (41.8%) in Spain and 1,019 (58.1%) in Colombia. The most frequent comorbidities were asthma (9.0%), chronic neurological disorder (NRL) (7.4%), immunosuppressive medication (7.2%), malignant neoplasms (5.4%) and chronic lung disease (not asthma) (CLD) (4.5%). Comorbidities associated with the different endpoints are summarized in Figure 1.

Conclusion: Asthma was associated with a significantly increased risk of death (OR: 4.17; 95%CI 1.34-12.97), MV (OR: 7.94 (3.59-17.56)), PICU admission (OR: 3.37 (1.91-5.96)), high flow/CPAP (OR: 6.65 (2.69-16.46)), and NP (OR: 3.85 (2.57-5.77)) compared to hospitalization without respiratory support. NRL was associated with increased risk of death (OR: 7.34 (3.01-17.90)), MV (OR: 3.07 (1.20-7.82)) and high flow/CPAP (OR: 4.36 (1.68-11.29)).

CLD was associated with increased risk of death (OR: 6.22 (2.28-16.94)) and NP (OR: 3.1 (1.74-5.38)) and in addition, chronic cardiac disease was associated with increased risk of MV (OR: 5.21 (1.76-15.41)) and PICU (OR: 2.78 (1.27-6.08)). Risks of death (OR: 4.49 (2.03-9.05)), MV (OR: 2.97 (1.52-5.81)), PICU (OR: 4.27 (2.89-6.33)), and NP (OR: 4.67 (3.64-5.99)) were higher in the Colombia Cohort.

Conclusion: Asthma, chronic neurological, cardiac and lung disease; and belonging to the Colombia cohort were consistently associated with multiple severe outcomes of COVID-19. Cancer and diabetes association with selected endpoints rather than with most endpoints may be more related to the baseline disease than with the actual COVID-19.
Multivariable association between comorbidities on the odds of each outcome (compared to hospitalization without respiratory support). Table 1. Color gradient across endpoints (red: risk factors, green: protective).

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic diabetes</td>
<td>1.36 (0.69-2.69)</td>
<td>0.352</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>1.18 (0.55-2.52)</td>
<td>0.682</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.19 (0.72-1.95)</td>
<td>0.494</td>
</tr>
<tr>
<td>Asthma, COPD, or emphysema</td>
<td>1.00 (0.81-1.23)</td>
<td>0.986</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.15 (0.83-1.58)</td>
<td>0.400</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>1.49 (1.06-2.08)</td>
<td>0.020</td>
</tr>
<tr>
<td>Neurological disease</td>
<td>1.46 (1.10-1.94)</td>
<td>0.008</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>1.76 (0.97-3.19)</td>
<td>0.061</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.20 (0.97-1.47)</td>
<td>0.093</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.66 (1.05-2.64)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

**836 PERSISTENT SYMPTOMS IN CHILDREN ADMITTED WITH COVID SIMILAR TO CONTROLS 1 YEAR LATER**

Alfredo Tagarro1, Sara Villanueva2, Ana Esteban Romero3, María López Luengo4, Marta Conde5, Lucía de Pablo6, David Aguilar-Alejano7, Itzi Gasteis8, Sara Dominguez9, Cristina Epalza10, Álvaro Ballestros11, Carlota Pinto12, Carlo Giaquinto13, Cinta Moraleda1

EPIICO, EPICD Colombia and ORCHESTRA

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**Background:** The aim of this study was to describe the prevalence of persistent symptoms of COVID in hospitalized pediatric population one year after admission compared to a control group.

**Methods:** Prospective observational study conducted in 2 hospitals. We included patients aged 0-18 years hospitalized for acute COVID-19 more than a year ago and controls, matched by age and sex, hospitalized for causes other than COVID-19, and who had never COVID-19 at recruitment or during the follow-up. Families were contacted and a standardized survey was conducted. Persistent COVID/disease was defined as the presence of symptoms with onset in the first 3 months after COVID-19 and with persistence for more than 2 months.

**Results:** 50 cases and 46 controls were analyzed. 58.3% male, 36% <5 years. Families were interviewed a median of 1.89 years (interquartile range: 1.25-2.07) after hospitalization. The definition of persistent COVID-19/disease was met in 34% of cases vs. 37% of controls (p=0.767). Symptoms persisted ≥11 months in 24% (12/50) of cases vs. 13% (6/46) of controls (p=0.182), with no differences by age group. The most frequent symptoms at 1 year in cases were fatigue (6%), headache (6%), poor appetite (6%), abdominal pain (6%) and variations in heart rate (6%). In controls, persistent symptoms were mostly abdominal pain (6%) and poor appetite (6%). The number of readmissions was 11/50 (22%) and 6/46 (13%) (p=0.267), respectively. On emotional/behavioral items, 16/50 (32%) of cases reported that their emotional state was worse or much worse than before admission, compared to 16/46 (34.7%). No risk factors associated with the development of persistent symptoms were found, except the length of hospital admission (p=0.043).

**Conclusion:** In this study, the prevalence of persistent symptoms was not different in patients with and without COVID-19. 1-year persistence was higher in COVID-19 cases but did not reach significance. Persistence correlated with length of hospitalization.

**837 PERSISTENCE OF SYMPTOMS ONE YEAR AFTER MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN**

Cinta Moraleda1, Sara Villanueva2, Olga Aldecoa Jiménez3, Blanca Díaz Fuentes4, Luis Prieto1, Daniel Blázquez2, Cristina Epalza9, Elsa Fernández Cooke10, Ángela Manzanares11, Pablo Rojo12, Serena Villaverde13, Álvaro Ballestros4, Sara Dominguez5, Alfredo Tagaro1

Hospital Universitario 12 de Octubre, Madrid, Spain, 1Pediatric Infectious Diseases Section. Pediatric Unit. University Hospital 12 de Octubre, Madrid, Spain, 2Pediatric Infectious Diseases Section. Pediatric Unit. University Hospital 12 de Octubre, Madrid, Spain, 3Complutense University of Madrid, Madrid, Spain, 4Fundación de Investigación Biomédica del Hospital Universitario 12 de Octubre, Madrid, Spain, 5Hospital Universitario 12 de Octubre, Madrid, Spain, 6Fundación para la Investigación Biomédica del Hospital Universitario 12 de Octubre, Madrid, Spain, 7University Europea de Madrid, Madrid, Spain, 8University of Padua, Padova, Italy

**Background:** The aim of this study was to describe the prevalence of persistent symptoms of COVID in hospitalized pediatric population one year after hospitalization compared to a control group.

**Methods:** This is a prospective observational study in under-18-aged patients diagnosed with MIS-C between October 2020 and April 2021 in a tertiary hospital. Data from initial episode was obtained from the Spanish national database and the medical history. A standardized phone questionnaire was done one year after the acute episode. As patients were aged and sex were included with i) history of acute COVID-19, from the same national database, and ii) with peritonitis diagnosis in the electronic medical record. Data was collected using REDCap and analyzed with R. Ethics committee approval was obtained.

**Results:** A total of 48 patients were included in the study. 16 in each group. Average age at hospitalization was 11.2 years old (IQR: 6.6-14.4) and 52% (23/48) were male. MIS-C patients presented high frequently 94% (15/16) cardiologic complications during hospitalization, in contrast with 19% (3/16) of acute COVID-19 patients and 25% (4/16) of peritonitis group (p=0.01). All of them resolved after a year except the ones associated to hypoxic ischemic encephalopathy in a patient with MIS-C that need ECMO assistance. Summary characteristics during acute episode are shown in Table 1. After one-year follow-up, 88% MIS-C patients suffered one or more symptoms, more frequently: headache (44%), fatigue (38%), insomnia (38%) and concentration problems (38%). A total of 56% of COVID-19 patients presented persisted symptoms, mainly fatigue and concentration problems (19%), and 31% in peritonitis group (19% loss of appetite and abdominal pain, p<0.001). MIS-C patients visited more frequently the medical professionals due to emotional change, behaviour or interpersonal relationships after the disease (4/16 (25%) in MIS-C vs. 0/16 (0%) in both control groups, p=0.028).

**Conclusion:** Majority of MIS-C patients have persistent symptoms one year after acute episode, even with the resolution of cardiologic complications. Frequency of long term symptoms in MIS-C patients is significantly higher than in COVID-19 hospitalized and than in a control group of surgical peritonitis patients.

Summary characteristics during acute episode

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MIS-C</th>
<th>COVID-19</th>
<th>Peritonitis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>11.2</td>
<td>11.2</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23/48</td>
<td>16/48</td>
<td>12/48</td>
<td></td>
</tr>
<tr>
<td>History of acute COVID-19</td>
<td>15/16</td>
<td>0/16</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>Persisted symptoms</td>
<td>37/48</td>
<td>15/48</td>
<td>4/16</td>
<td></td>
</tr>
<tr>
<td>Emotions/behavioral</td>
<td>16/48</td>
<td>0/16</td>
<td>0/16</td>
<td></td>
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<tr>
<td>Emotional/behavioral</td>
<td>16/48</td>
<td>0/16</td>
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<tr>
<td>Emotional/behavioral</td>
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<tr>
<td>Emotional/behavioral</td>
<td>16/48</td>
<td>0/16</td>
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</tbody>
</table>

**838 PBMC IMMUNOPHENOTYPING AND PLASMA INFLAMMATORY PROFILE OF CHILDREN WITH LONG COVID**

Jon Izquierdo-Pujol1, Sara Moron-Iopez2, Judith Dalmau3, Maria C. Puertas4, Clara Carreras-Abad3, Alba Gonzalez-Amatell3, Maria Mendez1, Carlos Rodrigo1, Javier Martinez Picado1

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**Background:** Long COVID can be developed by individuals after an infection with SARS-CoV-2 as described by the WHO. Although this condition is more commonly described in adults, it can occur in children and adolescents with a wide range of estimated prevalence of 1-25%. Little is known about the role of the immune system in long COVID. However, one of the main hypotheses about the underlying mechanism in long COVID is that there is an immune and inflammatory dysregulation that persists after the acute infection. The objective of this study is to compare immune cells populations, and inflammatory biomarkers in paediatric populations with and without long COVID.

**Methods:** We analyzed 55 blood samples from the pediatric COVID cohort (Hospital Germans Trias i Pujol), which includes more than 130 children diagnosed with long COVID and 23 controls. We measured different immune cell populations using spectral cytometry with a panel of 37 cellular markers, and 42 inflammatory markers using LumineX or ELISA. EdgeR was used for statistical analysis of the spectral data; p-values of inflammatory markers were calculated using the likelihood ratio test and they were corrected for multiple comparisons.

**Results:** The study cohort had a median age of 14.3 (IQR: 12.5-15.2) and 69.1% female. Patients had at least 3 symptoms associated with long COVID
839 INFLAMMASOME BUT NOT IFN-I/III RESPONSE IS ALTERED IN CHILDREN WITH LONG COVID

Matteo Fracella1, Leonardo Sorrentino, Federica Frasca2, Mirko Scordoio3, Alessandra D’Auria1, Giuseppe Oliveto1, Carla Selvaggi2, Greta Di Mattia3, Maria Giulia Conti3, Luigi Matera4, Domenico La Regina3, Gabriella D’Ettorre2, Guido Antonelli2, Alessandra Pierangelii, Carolina Scagnolari1.

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*Presented at CROI by a nonauthor colleague

Methods: Blood samples were collected from children attending Umberto I hospital of Rome, within 3–6 months after SARS-CoV-2 infection and from control children. RNA was extracted from PBMCs for determining the levels of IFN-I (IFN-Alpha2, -Beta, -Epsilon and -Omega), IFN-III (IFN-Lambda1-3), NLRP3 and IL-1beta genes, and miR-141 expression by quantitative RealTime-PCR assays, normalized to housekeeping GUS gene and RNU6B, respectively.

Results: 28 participants (M 12.5y SD 3.0) with LC symptoms, 28 participants (M 11.8y SD 3.0) without LC symptoms and 18 children who’ve never had SARS-CoV-2 infection (M 10.5y SD 3.1) were enrolled. Comparing the three study groups, reduced levels of IFN-Lambda1, IFN-Lambda2 and IFN-Lambda3 were found in LC patients (p< 0.001, p=0.012, respectively; we found reduced levels of IFN-Lambda1, IFN-Lambda2 and IFN-Lambda3 in children with and without long COVID.

Conclusion: The results of this study suggest that specific populations of peripheral blood immune cells might be involved in the mechanisms underlying prolonged COVID in children and adolescents. The increase in both IFN-Lambda1-3 and IgA CD21-22 memory B cells could be associated with the persistence of viral antigen in the gut and/or gut disbiosis. Moreover, the decrease in CD4+ T cells could be related to autoantibodies against G-protein coupled receptors (GPCRs), since this cell population can express GPR56, and autoantibodies against GPCRs were previously reported to be elevated in adults with long COVID.

840 PROTECTION AGAINST HETEROLOGOUS CHALLENGE 1 YEAR AFTER INFANT SARS-CoV-2 IMMUNIZATION

Emma C. Milligan1, Katherine Olstad2, Caitlin Williams3, David Montefiori4, Michael Hudgens5, Darin Edwards6, Andrea Carfi7, Rizzizzeria Corbett5, Christopher Fox5, Mark Toma5, Rachel Reader2, Dirk Dittmer1, Koen Van Rompay1, Sallie R. Permar3, Kristina De Paris1

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Background: Although mRNA-SARS-CoV-2 vaccines have received emergency-use authorization for infants age 6 months and older, vaccine uptake is slow, stressing that questions of safety and durability of vaccine efficacy remain prominent.

Methods: Infant rhesus macaques (RM) (n=8/group) at 2 months of age, comparable to human toddler age, were immunized intramuscularly at weeks 0 and 4 with 30μg stabilized prefusion SARS-CoV-2 S 2P spike (Protein) (Washington strain) encoded by mRNA encapsulated in lipid nanoparticles (mRNA-LNP) or 15μg S protein mixed with 3M-052 in stable emulsion (Protein). At 1 year, vaccinated and age-matched unvaccinated RM (n=8) were challenged intranasally (10^10pfu) and intratracheally (2x10^9pfu) with B.1.617.2. Lung radiographs and pathology were blindly assessed, viral RNA gene (vRNA) copies were measured by qPCR in pharyngeal swabs and lung, and neutralizing antibody and peripheral blood T cell responses were measured.

Results: At 1 year, DE14G-specific neutralizing antibody (nAb) titers were still detectable in the Protein group (ID50 =75; range: 359-1,949) and mRNA-LNP groups (ID50=73; range: 41-240). Both vaccines also induced cross-neutralizing antibodies to B.1.617.2. Peripheral blood CD4+ T cell responses to the ancestral spike protein at week 52 did not differ between the groups. However, median CD8+ T cell responses were higher (p=0.002, Mann Whitney) in the mRNA-LNP group (2.8%; range: 0.9%-7.1%) compared to the Protein group (0.8%; range: 0.1%-1.6%). Control RMs had significantly higher median vRNA copies/ml (1.4±2.7x10^10) in day 4 pharyngeal swabs compared to Protein (3.8±6.8x10^9) or mRNA-LNP (4.4±9.7x10^5) vaccinated RMs. Severe lung pathology was observed in 7 of 8 controls compared to 1 of 8 or 0 of 8 RMs in the mRNA-LNP or Protein group respectively. Protection against lung inflammation was associated with nAb titers (r=-0.592, p=0.003) (Figure 1).

Conclusion: These results demonstrate that despite lower vaccine doses compared to adults, both protein and mRNA vaccines were safe, induced durable immune responses and provided comparable protective efficacy against infection with a heterologous SARS-CoV-2 variant in infants, implying that early life vaccination of human infants may lead to durable immunity. Neutralizing ID50 antibody titers are a correlate of protection in infant RMs challenged with SARS-CoV-2

841 mRNA VACCINES INDUCES A HIGHER ANTIBODIES RESPONSE IN CHILDREN WITH PREVIOUS COVID-19

Costanza Di Chiara1, Anna Cantarutti1, Francesco Bonfante1, Maria Raffaella Petrara1, Chiara Cosma1, Matteo Pagliari2, Nicola Cotugno1, Alessandra Meneghel1, Elisa Benetti1, Luca Bosa1, Paolo Palma4, Anita De Ross1, Carlo Giaquinto4, Daniele Dona1, Andrea Padoan1

1University of Padova, Padova, Italy, 2University of Milan, Milan, Italy, 3Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy, 4Bambino Gesu Children’s Hospital, Rome, Italy

Background: mRNA vaccines elicit a durable humoral response to SARS-CoV-2 in adults, whereas evidence in children is lacking. This study aimed to evaluate
the early and long-term immunological response after the BNT162b2 vaccine in children with or without a previous SARS-CoV-2 infection.

Methods: In a multicenter, prospective, observational study we profiled the immune response to the BNT162b2 vaccine in children aged 5–11 years attending the Pediatric Departments at the University of Padua and Bambino Gesù Children’s Hospital in Rome (Italy). Forty-four healthy children (HC), 20 immune compromised (IC), and 18 children who previously developed MIS-C (MIS-C) were included in the study. Blood samples were collected pre-, 1, and 6 months after a 2-doses vaccination schedule. Neutralizing antibodies (NAb) and anti-S-RBD IgG titers were analyzed through plaque reduction neutralization test (PRNT) and chemiluminescent immune–enzymatic assay (CLIA), respectively. B and T cell phenotypes were analyzed by flow cytometry. Geometric mean titers (GMTs) and 95% confidence intervals and median and interquartile range (IQR) of variables were evaluated according to pre-existing confirmed COVID-19.

Results: Eighty-two children were studied; 60 with a molecular-documented previous COVID-19 (Group A) and 22 without previous defined as the absence of antigen-specific antibodies before the vaccination (Group B). Overall, in Group A we observed higher NAb GMTs, anti-S-RBD titers, and T- and B-reg cells than in Group A, at both 1 and 6 mo after vaccination (table); NAb against the parental virus resulted to be greater in Group A. As in Group B by a factor of 18 and 11, at 1 and 6 mo after vaccination, respectively. Both groups recorded a decrease in antibody titers of approximately 50–70% between 1 and 6 months. A significant difference for Omicron NAb (p = 0.02) and anti-S-RBD (p = 0.07) titer decay was observed between Group A and B. In contrast, Parental NAb titers appeared to have similar trends in the 2 groups (p = 0.47). Comparable antibody titers at 1 and 6 mo. (p = 0.37) were detected across the three categories of HC, IC, and MIS-C (table).

Conclusion: mRNA vaccination triggers a higher humoral response in children with a previous history of COVID-19, regardless of the immune deficiency or previous MIS-C, at least up to 6 mo, providing insight into boosting preexisting immunity with mRNA vaccines.

Table: Humoral and cell response comparison between groups A and B, overall, and stratified according to HC, IC, and MIS-C.

EFFECT OF BNT162B2 ANTIGEN DOSAGE ON PROTECTION AGAINST SARS-CoV-2 OMICRON INFECTION
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Background: Coronavirus Disease 2019 (COVID-19) vaccine antigen dosage may affect protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, but direct evidence to quantify this effect is lacking.

Methods: A matched, retrospective, cohort study that emulated a randomized controlled trial was conducted in Qatar between February 3, 2022 and November 8, 2022, to provide a head-to-head, controlled comparison of protection induced by two antigen dosages of the BNT162b2 vaccine. The study compared incidence of omicron infection in the national cohort of adolescents 12 years of age who received the two-dose primary-series of the 30-µg BNT162b2 vaccine to that in the national cohort of adolescents 11 years of age who received the two-dose primary-series of the pediatric 10-µg BNT162b2 vaccine. Associations were estimated using Cox proportional-hazard regression models.

Results: Among adolescents with no record of prior infection, cumulative incidence of infection was 6.0% (95% CI: 4.9–7.3%) for the 30-µg cohort and 7.2% (95% CI: 6.1–8.5%) for the 10-µg cohort, 210 days after the start of follow-up. Incidence during follow-up was dominated by omicron subvariants including, consecutively, BA.1/BA.2, BA.4/BA.5, BA.2.75, and XBB. The adjusted hazard ratio comparing incidence of infection in the 30-µg cohort to the 10-µg cohort was 0.77 (95% CI: 0.60–0.96). Corresponding relative effectiveness was 23.4% (95% CI: 1.6–40.4%). Relative effectiveness was -3.3% (95% CI: -68.0–27.5%) among adolescents with a record of prior infection.

Conclusion: Three-fold higher BNT162b2 dosage was associated with ~25% higher protection against infection in infection-naïve adolescents of similar age. These findings may inform design of future COVID-19 vaccines and boosters for persons of different age groups.

STUDY ON THE FREQUENCY OF DUAL INFECTIONS IN NEW HIV-1 DIAGNOSES IN SPAIN
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Background: Dual infections (DIs) with phylogenetically-distinct HIV-1 strains have been reported. DIs can lead to generation of novel recombiant strains and may be associated with faster disease progression. Here we estimate Di frequency in Spain among newly diagnosed HIV-1 infections and examine associated epidemiological, virological, and clinical factors.

Methods: Plasma samples were obtained from 498 patients with HIV-1, antiretroviral drug-naive, and newly diagnosed in 2017–2020 in 6 Spanish regions. Reverse transcriptase (RT) and env V3 region fragments were amplified by RT-PCR/nested PCR from plasma-extracted RNA. Amplicons were sequenced with illumina MiSeq 2x300 sequencing protocol. Ultra-deep sequencing (UDS) data were analyzed with a home-developed pipeline. Only samples with ≥500 sequences in both segments were used in further analyses. Sequences were grouped according to similarity (97%) in operational taxonomic units (OTUs). Phylogenetic trees were constructed including all OTUs and all RT (n = 21,777) and V3 (n = 8,268) Sanger sequences obtained in our laboratory. DIs were identified as those with OTUs branching separately in either RT or V3, interspersed among sequences from other patients, with sequences from the minor variant comprising ≥0.5% and ≥5% sequences. Clinical and epidemiological associations were evaluated using chi-square (categorical variables) and Mann-Whitney (continuous variables) tests, and logistic regression.

Results: DIs were detected in 23 (4.6%) patients, of which 10 were in subtypes B, 1 CFR02_AG and 13 were intersubtype (all B + non-B). 14 DIs were detected in RT, 5 in V3, and 4 in both fragments. Non-subtype B infections and infections belonging to transmission clusters of ≥4 individuals were significantly associated with DIs. Mixed bases in protease–RT previously identified in Sanger sequencing were significantly more numerous in DIs (mean 20.1 vs. 5.1). Age, gender, CD4+ T-cell counts, and viral load were not significantly associated with DIs, and there was a marginally non-statistically significant tendency for more frequent DIs in men who have sex with men and persons who inject drugs.

Conclusion: Dual infections were detected in 4.6% of newly diagnosed HIV-1 infections in Spain, more frequently among non-B and clustering infections. To our knowledge, this is the largest reported study on dual infections based on UDS. Information on HIV-1 DIs may have important clinical, epidemiological, and public health implications.

EPIDEMIOLOGICAL AND MOLECULAR EVOLUTION OF THE HIV-1 CRF94: BIRTH OF CRF132
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Background: In 2018 we reported the emergence of the new HIV-1 recombinant CRF94_02B2 involved in a large transmission cluster of 49 French MSM mostly infected in 2016-2017. This CRF94 raised concerns of enhanced virulence. Prevention actions were undertaken in the area and population affected. This study reported the molecular and epidemiological evolution of this CRF94 until June 2022.
Methods: In 2021-2022, French sequence databases were screened for patients infected with HIV-1 subtype CRF94 or similar strain. HIV subtyping was confirmed by phylogenetic analysis of genes encoding both protease and reverse transcriptase (1070bps), and integrase (696bps) using IQ-TREE. Five whole genomes, related but distinct from CRF94, were obtained with the DeepChek™ assay Whole Genome kits. Recombination breakpoints were estimated using RDP4 and SimPlot. Mann-Whitney and LogRank tests were used for statistical analyses to compare patients' characteristics.

Results: In June 2022, 49 new HIV-1 sequences were collected: 14 clustered with the 49 previous CRF94, 32 formed a new cluster next to but distinct from CRF94, and 3 strains could not be classified. Analysis of 5 whole genomes from the new cluster revealed a new recombinant, the CRF132_94B, mainly consisting of CRF94 with recombinant subtype B in the POL and accessory genes. Vif gene changed from the F2 to the B subtype. Both CRF94 and 132 clusters involved >95% of NSM, mostly infected < 1 year before diagnosis. However, there were differences: 97% were diagnosed in 2013-2019 for CRF94 vs 90% in 2020-2022 for CRF132. At time of diagnosis, 33% of patients infected with CRF94 knew the Prep vs 95% for CRF132. In the cluster CRF94, patients were older (34 vs 30 years, p = 0.02), had higher viral loads (5.42 vs 4.42 log10 copies/mL, p < 0.001), a lower CD4 cell counts (358 vs 508/mm3, p = 0.002). On treatment, the patients with the CRF94 reached viremia < 50 copies/mL vs > 500 copies/mL in 77.5% to 77.5% and specificity from 89.9 to 92.3%.

Conclusion: The accuracy of inference of directionality and linkage in a large epidemic using molecular epidemiology is lower than consensus sequences are used. However, accuracy increases considerably if additional epidemiological data leading to a high pre-test probability are available, and NGS data are used. Precision-Recall Curves with Varying Parameters

846 PRESENCE OF CXCR4- USING HIV-1 VIRUS IN NEWLY DIAGNOSED INDIVIDUALS IN NORTH CAROLINA
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Background: HIV-1 uses either CCR5 or CXCR4 as the coreceptor to enter host cells. Transmitted viruses are usually CCR5-using (R5 virus), and CXCR4-using (X4/dual) viruses emerge in the late stage of disease. In 2018 we implemented a statewide recency assay for HIV-positive tests conducted at the NC public health lab. This platform uses a UMI-based NGS approach to sequence portions of the HIV-1 genome including the env gene V3 region for co-receptor prediction. We assessed the prevalence of X4 viruses among people with newly diagnosed HIV-1 from 2018 to 2021.

Methods: Remnant diagnostic sera from people with new HIV-1 diagnoses were sequenced using the Multiplexed Primer ID MiSeq protocol targeting the HIV-1 pol/gene and env V1-V3 region. Recent HIV-1 infections (within 9 months of infection) were estimated based on pairwise sequence diversity. Coreceptor tropism was predicted using Geno2Pheno algorithm using two false positive rate (FPR) cut-offs of 2% and 5.75%.

Results: A total of 726 people had sufficient sequence depth for the analysis. Overall, using the 2% and 5.75% cutoffs, we found that 10.3% and 21.5% had X4 variants, with a median abundance within the individual viral populations of 32.3% (IQR: 10.9-85.2%) and 53.3% (IQR: 11.5-97.6%), respectively. Using the 9-months recency cut-off, we found significantly more X4 variants in the chronically infected individuals (13.8 and 26.8%) than the recently infected (5.9 and 15.6%) using both FPR cut-offs (p < 0.01). Most of the X4 variants found in the recent infections had homogenous populations, with a median abundance greater than 95% at both cut-offs, suggesting they were transmitted X4 variants. Conversely, most chronically infected individuals had mixed populations of R5 and X4 viruses, consistent with a late diagnosis of infection. Among the recently infected patients, those with X4 variants had significantly lower CD4 counts using 2% FPR cut-off (p < 0.01). Carrying X4 variants was not associated with having significantly more DRMs at the pol region.

Conclusion: We previously observed that viruses with an FPR≤2% have an X4 entry phenotype while half of those with an FPR from 2% to 5.75% have an X4 entry phenotype. Thus, we estimate that the overall transmission rate of X4 viruses is approx. 10%. This is significantly higher than previously thought and may represent further maturation of the epidemic with an increased fraction of transmissions from chronically infected people not on therapy.

845 ACCURACY OF MOLECULAR EPIDEMIOLOGICAL INFERENCE IN A SIMULATED EPIDEMIC
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Background: Molecular Epidemiology is the 4th pillar of the Ending the HIV Epidemic plan, and it is hoped that the use of these analyses will lead to both prevention and treatment interventions in at-risk populations. Concern has been voiced that the inferences derived from molecular epidemiological (ME) analyses could be used in criminal and civil litigation. However, the level of concern is impacted by the accuracy of these analyses. Here we assessed the accuracy of ME analyses to identify correct transmission pairs and the correct direction of transmission in a simulated HIV epidemic.

Methods: An agent-based simulation based on the EpiModel software package was parameterized to fit the San Diego HIV epidemic among MSM. The simulated data, which included information of who transmitted to whom and the time of transmissions, were used to simulate HIV genetic sequences for the corresponding individuals, incorporating time from infection to sampling. Both full length consensus and next generation sequences (NGS) were generated. These sequences were then analyzed to measure the ability of consensus and NGS to predict correct linkage and directionality. We then repeated the analyses varying sample fraction, portion of genome sequenced, human migration in and out of the region of interest, and the inclusions of assays for testing recency of infection when inferring who infected whom.

Results: Consensus sequences did not reliably predict correct directionality and linkage, regardless of sampling depth. See Figure 1. For NGS data, given the intense computation burden associated with analyzing these sequences, we chose pairs of individuals where the sequence data had a high likelihood of correctly identifying both linkage and transmission direction (infect 90% of 80%). Within this subset, we found a sensitivity that ranged from 67.5 to 77.5% and specificity from 89.9 to 92.3%.
HOTSPOTS OF RECENT HIV INFECTIONS AMONG ADULT PEOPLE LIVING WITH HIV IN NIGERIA
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Background: In Nigeria, rectency testing was introduced in 2020 to assist in identifying those who had recently acquired HIV infection between 4-24 weeks and 12 months so that prevention programs can focus on preventing incident infections and ultimately break the chain of transmission to control the epidemic. This study aimed to identify the spatial distribution pattern of recent HIV infection among adult PLHIV, identify correlates of recent HIV infection and detect recent HIV infection hotspots.

Methods: This was an ecological descriptive and analytical design conducted between October 2019 and December 2021. We georeferenced recent HIV case reports from 197 of 774 Local Government Areas (LGAs) spread across all of Nigeria’s six geographic regions. Percent of recent HIV infections among adult PLHIV was calculated from data extracted from the national Recency testing registers. Spatial autocorrelation of recent HIV infection was analysed via a Global Moran’s I test. Hotspot analysis using Getis-Ord Gi* statistics highlighted regions of significant clusters of recent HIV infection among neighbouring geographic regions, and Poisson regression model identified correlates of recent HIV infection in Nigeria.

Results: Among the 62,382 PLHIV tested for recent infection, 61% were females. Of the 4,077 HIV PLHIV identified as having recent HIV infection, 1,499 (6.3%) were males and 2,578 (6.7%) were females. Females between the ages of 15–19 (10%) had the highest recent infection when compared to other female age groups, while males (16%) aged 50 and over were four times more likely than females (4%) in this age group to have recently contracted HIV. The global spatial autocorrelation Moran’s I statistics (z-score = 10.7, p < 0.0001) revealed a clustered distribution of the spatial pattern of recent HIV infection. Hotspot analysis identified significant clusters of recent HIV infection that were confined to LGAs in the North Central, North East, South West, South East and the South South regions of the country. In the Poisson model, knowledge of HIV prevention (p < 0.0004) and ART coverage (p < 0.0001) were significantly associated with recent HIV infection.

Conclusion: Given the extent of knowledge of HIV prevention and ART coverage on recent HIV infection among adult PLHIV, adoption of policies and strategies to enhance awareness and encourage people to seek early HIV testing and implementation of intensified community ART delivery services can break the transmission of HIV infection.

Hotsps of Recent HIV Infections, October 2019 and December 2021

ANALYSIS OF HIV RECENT INFECTION SURVEILLANCE DATA AMONG YOUNG PEOPLE IN MALAWI
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Background: Malawi has made significant progress towards UNAIDS 95-95-95 targets, yet awareness of HIV status is at 88.3% and lowest among young people, 15-24 year-old (76.2%). Low awareness of HIV status may contribute to further transmission in the population, therefore there is a need to better understand HIV testing history and transmission in this age group.

Methods: We analyzed pooled recent HIV infection data to describe testing history and recent HIV infection (< 12 months) status among 8,389 newly HIV-diagnosed 15-24-year-olds from 251 sites across Malawi, between September 2019 – March 2022. HIV recent infection classification was based on positive rapid test for recent infection and a viral load ≥ 1000 copies/ml. We calculated the proportion of consenting newly HIV-diagnosed participants classified with
Recent HIV infection by age, sex, residence, testing point, and self-reported testing history to describe differences related to risk and behavior.

**Results:** Most newly HIV-diagnosed young people were female (84.1%), aged 23–24 years-old (32.1%), rural residents (60.4%), and diagnosed at voluntary counselling and testing points (53.8%). A history of reported prior HIV testing was less frequent in younger age groups (Figure 1). Among 15–24-year-olds, 32.9% of males and 16.1% of females reported no previous HIV testing history. Overall, 4.9% of new HIV diagnoses were classified as recent infections, with the highest proportions observed in Mzimba (8.5%) and Machinga (6.9%) districts, among breastfeeding women (8.2%), persons tested at sexually transmitted infection clinics (9.0%), persons with a prior test within the past 6 months (11.9–13.5%), and 17–18-year-olds (7.3%).

**Conclusion:** These findings highlight gaps in HIV testing among young people by age and sex with the majority (>95%) potentially having been infected for >12 months. Tailored and innovative HIV prevention and testing strategies for adolescents, young males, and pregnant and breastfeeding women may be needed for HIV epidemic control. Routine data collection and analysis of recent HIV infection data and triangulating various surveillance data sources can be utilized to inform targeted HIV testing and preventive strategies for young people.

Figure 1: The age/sex disaggregated proportion of young people with a positive HIV test and their reported time of last HIV test.

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**850 HIV RECENT INFECTION IN SEXUAL AND GENDER MINORITIES IN BRAZIL AND PERU: ImPReSt STUDY**


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**Background:** HIV incidence estimation is critical for monitoring the HIV epidemic dynamics, transmission trends and effectiveness of public health prevention interventions. We aimed to identify HIV recent infection (RI) cases, its associated factors and to estimate annualized HIV incidence using recency testing among sexual and gender minorities (SGM) undergoing HIV testing in Brazil and Peru.

**Methods:** Cross-sectional multicenter study in HIV testing services in Brazil (6 cities) and Peru (7 cities) from Jan/21–May/22. Inclusion criteria: 18+ years, cisgender men who have sex with men (cis-MSM), transgenders (TGW) and other SGM. Exclusion criteria: HIV+, current PrEP/PPE use. Rapid HIV 4th generation test was used; dried blood spots (DBS) were obtained from the HIV+ cases. We identified HIV RI using the Maximum HIV-1 Lag-Avidity EIA assay as part of RI testing algorithm (RITA), which includes HIV-1 RNA < 400 copies/mL, CD4 count < 200 cells/mm³, and prior/current ART use (to exclude long-standing infections). We assumed mean duration of recent infection of 214 days (95%CI: 193-237) and a false recent ratio (FRR) of 0%. Annualized HIV incidence was calculated per country using the WHO mathematical formula. Multivariable logistic regression models per country were used to estimate factors associated with HIV RI.

**Results:** Of 7362 individuals approached, 7116 (97%) were eligible and enrolled (Brazil: 4700 (66%); Peru 2416 (34%)); 86% cis-MSM, 11% TGW, 3% other SGM. Median age was 27 years (IQR:23-34), 64% ≤ 30 years, 72% secondary education, 74% low income. In the prior 6 months, 35% reported >5 sex-partners, 79% condomless sex, 20% STI symptoms, 14% sex work, 27% substance use and 57% binge drinking. HIV prevalence was 13.7% (N=971; Brazil: 470/4700 (10.0%); Peru: 501/2416 (20.7%)). DBS were available for 959 (99%); 165 (17.2%) were classified as recently infected (Brazil: 87/4700 (1.7%); Peru: 84/2416 (3.5%)). The overall annualized HIV incidence rate was 4.64% (95% CI: 4.11-5.17; Brazil: 3.30% (95% CI: 2.76-3.84); Peru: 7.59% (95% CI: 6.40-8.78). Multivariable models showed that in both countries engaging in condomless sex increased the odds of HIV RI and in Peru being 30 years or younger (see Table).

**Conclusion:** High levels of HIV prevalence, HIV RI and annualized HIV incidence among SGM in Brazil and Peru highlight the burden of the HIV epidemic among these populations. Public Health policies and interventions to increase PrEP access in Latin America are urgently needed.

Factors associated with HIV recent infection among sexual and gender minorities from Brazil and Peru: ImPReSt Seroincidence Study.

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**851 HIV TIME-SPACE ALERTS AMONG PWID AND MSM IN THE UNITED STATES, 2018-2021**


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**Background:** HIV time-space (TS) cluster detection is routinely conducted by CDC and by state and local health departments to identify U.S. counties where diagnoses are elevated compared with historical levels. These analyses generate “alerts” for review that may indicate clusters or outbreaks needing response. Previously, national TS cluster detection primarily focused on persons who inject drugs (PWID). Differences in the frequency, characteristics, and recurrences of alerts among men who have sex with men (MSM) versus alerts among PWID have not been previously described.

**Methods:** HIV diagnoses reported to the National HIV Surveillance System were analyzed quarterly during 2018–2021 to detect TS alerts using standard CDC methods. Each quarter, for each county, the number of HIV diagnoses during the preceding 12 months among the group of interest (PWID and MSM) was compared with the annual average from the preceding 3 years. An alert was generated if the number of diagnoses in the year of interest was >2 standard deviations and >2 diagnoses above the baseline number of diagnoses. Within each alert type — PWID and MSM — alerting counties were stratified by urban-rural classification. For counties with initial alerts during 2018-2019, recurrences of alerts during the following 8 quarters were identified.

**Results:** During 2018-2021, PWID alerts occurred in 154 (4.9%) counties and MSM alerts occurred in 445 (14.2%) counties (Table). PWID alerts occurred in a higher percentage of large central metro areas (41.2%) than other areas (0.3–14.2%). MSM alerts occurred in a higher percentage of large fringe (29.1%) and medium (27.4%) metro areas than other areas (3.7–19.6%). Multiple subsequent alerts were more common for PWID than for MSM alerts: 30% of counties with PWID alerts had ≥2 subsequent alerts in the following 8 quarters, compared with 19% of counties with MSM alerts.

**Conclusion:** The occurrence of PWID alerts in >41% of large central metro areas is concerning, as it might suggest expanding HIV transmission among PWID in these areas. Application of similar time-space alert criteria to assess TS clusters among MSM yields nearly three times as many alerts among MSM as among PWID. Compared with PWID alerts, a lower percentage of MSM alerts recurred, suggesting that increases detected typically are not sustained. Additional work to refine TS cluster detection criteria, or to prioritize additional follow-up or investigation, is needed for MSM alerts.
Table. Urban-rural classification* of counties with time-space alerts, 2018–2021

<table>
<thead>
<tr>
<th>Type of clusters</th>
<th>Time periods (n clusters)</th>
<th>Median age</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2013-15/2016-18/2019-2021</td>
<td></td>
<td></td>
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<tr>
<td>Not in a cluster</td>
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<td></td>
</tr>
<tr>
<td>Small cluster (2-4)</td>
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<tr>
<td>Large cluster (&gt;8)</td>
<td></td>
<td>44.6</td>
<td>15.5</td>
</tr>
</tbody>
</table>

*Urban-rural classification: urban = n = 200, semi-rural = n = 120, rural = n = 100.
HIV SEQUENCING AT DIAGNOSIS ENHANCES DETECTION OF CLUSTERS AND POTENTIAL CARE GAPS

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Background: HIV genetic sequencing enables identification of growing transmission clusters, but typically occurs after a person diagnosed with HIV is linked to care and undergoes drug resistance testing. Many surveillance systems utilize these sequences to assess for outbreaks but could miss members who have not engaged in clinical care. We assessed whether additional sequencing from remnant diagnostic specimens could enhance cluster detection and response activities in a statewide surveillance system.

Methods: We built an analysis platform to monitor HIV clusters in North Carolina (NC). To date, >20,000 pol sequences from routine “clinical” genotypes have been systematically reported to state surveillance from reference laboratories. From 2018 to 2021, we performed next-generation sequencing (NGS) on remnant diagnostic specimens from individuals with new HIV diagnoses at the NC State Laboratory for Public Health to augment molecular surveillance. We evaluated clinical and cluster characteristics of persons who had subsequent clinical sequences compared to those who only had NGS at diagnosis. Clusters were defined at <1.5% genetic distance threshold between ≥2 sequences using the TN-93 model, including sequences from persons diagnosed prior to 2018.

Results: In total, 855 persons newly diagnosed with HIV had NGS from remnant specimens; 591 (69%) had a subsequent clinical sequence reported to surveillance and 264 (31%) had NGS only. Of persons with clinical sequences, 89% had at least one care visit (≥1 recorded viral load or CD4 cell count) and 73% were virally suppressed in 2021. Among persons with only NGS, 83% had linked to care since diagnosis, 69% had at least one care visit in 2021, and 55% were virally suppressed in 2021. Persons with only NGS were less likely to be identified in a cluster (62% vs. 74% with a subsequent sequence). There were 319 unique clusters (median size: 5 members; Range: 2-104); 122 (38%) clusters included ≥1 newly diagnosed member with only an NGS (median: 1 member [Range: 1-5]). In 22 (7%) clusters (size: 2-6), all new diagnoses had only NGS.

Conclusion: Almost a third of individuals newly diagnosed with HIV did not have a clinically reported HIV sequence following diagnosis. These persons were somewhat less engaged in care, and over half were linked to HIV clusters, including membership in over one-third of clusters. HIV sequencing at the time of diagnosis enhances cluster detection, highlighting areas where intervention and monitoring could be intensified.
857 CHANGING RISK OF HIV BEHAVIORAL CLUSTERS IN RAKAI, UGANDA
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Background: New HIV infections have declined substantially in eastern Africa with the introduction of combination HIV interventions (CHI). However, it is unknown whether incidence has declined throughout the population or whether ongoing transmission is driven by sub-populations with high HIV incidence.
Methods: We used a machine learning algorithm applied to 12 survey rounds of data (1999-2016) from the Rakai Community Cohort Study (RCCS) in Uganda to identify population sub-groups with similar demographic and sexual behavioral characteristics. Sub-groups (i.e., clusters) were identified using k-means clustering agnostic to HIV status, with the number of clusters selected to optimize parsimonious prediction of HIV status. We assessed changes in cluster size, proportion of incident HIV cases accounted for, and within-cluster HIV incidence rates over time. Our algorithm supported 3- and 36-cluster solutions, depending on the fit statistic used (BIC vs. AIC). HIV incidence rate ratios (IRR) and 95% confidence intervals were calculated by visit and CHI epoch (pre CHI: 1999–2004; early CHI: 2005–2011; late CHI: 2011–2016) for each cluster. In the 3-cluster solution, we categorized clusters as low (0.38/100 person years [pys]), average (0.79/100 pys), and high (1.15/100 pys) risk based on pre-CHI HIV IRRs.
Results: 33,866 individuals contributed 102,759 person-visits to the analysis. Most clusters with low pre-CHI incidence increased in size while the size of average and high-risk clusters decreased or remained unchanged. In the 3-cluster solution, the low-risk cluster increased from 8.7% of participants in 1999 to 20.0% by 2016, while the average-risk and high-risk clusters decreased in size from 50.9% to 45.3% and 40.3% to 34.5% respectively. IR per 100 py trending downward in low and medium risk clusters, while significantly declining in the high-risk cluster (IRR 0.50, 0.29-0.86). In the 36-cluster solution, HIV IR declined in most clusters (Figure). The majority of incidence reduction (89%) could be attributed to within-cluster risk reductions, rather than changes in cluster size. Incidence did not concentrate among clusters with higher pre-CHI incidence over time.
Conclusion: In southern Uganda, HIV incidence has declined in nearly all population sub-groups with few exceptions and with no concentration of incidence in high-risk groups. More targeted strategies may be needed to reduce HIV risk in population sub-groups where incidence has remained stable or increased.
Changes in cluster incidence rate, size, and attributable fraction of incident HIV cases over time (36-Cluster Solution). Asterisks indicate clusters where there were zero incident cases in either the early or late-CHI period. Clusters are numbered in ascending order based on pre-CHI incidence levels (e.g., cluster 1 had the lowest pre-CHI incidence and cluster 36 the highest).

859 TRANSGENDER WOMEN BASELINE PROFILE IN TRANSICTAR: TRANS-SPECIFIC COHORT IN ARGENTINA
Claudia E. Frola1, Romina Caballero, Carina César, Emilía Frontini, María I. Figueroa, Carolina F. Pérez, Nicolás Doudchtzky, Nadir Cardozo, Virginia Zalazar, Ana Gun, Pedro E. Cahn, Valeria Fink, Inés Aristegui
TransICTAR Study Group
Fundación Huésped, Buenos Aires, Argentina
Background: Transgender women (TGW) are among the population most affected by the HIV epidemic in Argentina, despite a progressive legal framework. TransICTAR is a trans-specific cohort in Argentina that aims to assess physical and mental health among transgender and non-binary people (TGBNP). We present baseline characteristics of TGW.
Methods: TGW attending a trans-friendly clinic to receive HIV/STIs prevention/treatment, mental health care and/or gender-affirming hormone therapy (GHT) were invited to participate. Semiannual visits including clinical assessments, laboratory tests, and psychosocial interviews were performed. Oral PrEP was offered as part of a combined prevention strategy since September 2021.
Results: Between September/2019 and August/2022, 500 TGNBP were enrolled, 416 were TGW (median age: 30 years, IQR 25–37). High social vulnerability was observed (Table 1). Regarding trans-specific characteristics, 49.8% reported industrial silicone injections and 36.8% were receiving GHT. 76.9% were sex workers. Baseline STIs prevalence were: HIV 42.3% (10.2% diagnosed at enrolment), syphilis 40% (defined as positive nontreponemal test VDRL with titers of at least 1/2), past HBV 18.5%, chronic HCV 3.8%, HCV antibody positive 2.6%. Only 57% presented HBV protective antibodies titers (HBsAg<10U/l/ml), 8 TGW were on PrEP. For those with HIV, median CD4+ cell count was 602 cells/mm3 (IQR 378-933), 66.5% were on ART at enrolment (53.6% were virally suppressed) and 14.8% initiated at baseline. During 36 months of follow up, 4 TGW died (one AIDS-related and one COVID-19-related). Bivariate analyses showed that a positive HIV diagnosis was independently associated with migration, low level of education, unstable housing, silicone injection and sex work, while was negatively associated with being on GHT. In multivariable logistic regression, only sociodemographic variables remain associated: migrant (aOR=.487, 95% CI=[0.34–0.76]); incomplete high school (aOR=.463, 95% CI=[0.30–0.71]); unsteady housing (aOR=.614, 95% CI=[.401–.940]); and sex work (aOR=.324, 95% CI=[.177–.593]).

858 SEX DIFFERENCES IN NON-FATAL OVERDOSES: A POPULATION-BASED COHORT STUDY
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1Columbia University, New York, NY, USA, 2British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada, 3Simon Fraser University, Vancouver, BC, Canada
Background: Canada and the United States continue to face increases in both fatal and non-fatal overdoses. In Canada, the magnitude of the overdose crisis is most severe in British Columbia (BC), with sex disparities among people experiencing fatal overdoses higher than in any other province. However, little is known about sex disparities pertaining to non-fatal overdoses (NFOD) among people living with HIV (PLWH).
Methods: We used data from the Comparative Outcomes and Service Utilization Trends (COAST) study, a population-based longitudinal cohort study in BC, Canada. Administrative health records are available for all known PLWH and a 10% random sample of the HIV-negative population, all ≥19 years of age. Using data from January 1st, 2012 – March 31st, 2020, we identified 12,717 PLWH and 473,947 people living without HIV. We defined NFODs using ICD-9-10-C codes in linked administrative data sets (i.e., hospital admissions, emergency department, and physician visits).
Results: Within COAST, 21.8% (n=2767) of PLWH and 49.7% (n=235,655) of the HIV-negative group are female. Among females living with HIV (FLWH), 18.3% (n=508) experienced at least one NFOD, compared to 10.7% (n=1069) of males living with HIV, 1.3% (n=3043) of females and 1.5% (n=3473) of males living without HIV. FLWH who experienced an initial NFOD had, on average, 2.5 recurrent NFODs; males living with HIV had, on average, 1.8. In contrast, males and females in the HIV-negative comparison group had <1 recurrent NFOD. Overall, FLWH had the highest NFOD incidence (10.53/100 person-years [PY]; 95%CI: 10.05, 11.03). These rates surpassed those of males living with HIV (4.86/100 PY; 95% CI: 4.65, 5.04), and females (0.37/100 PY; 95%CI: 0.36, 0.38) and males (0.44/100 PY; 95% CI: 0.43, 0.45) living without HIV. In unadjusted analyses, FLWH were more likely to experience an NFOD than men living with HIV, with IR declining (IRR: 2.17; 95%CI: 2.04, 2.30), whereas females living without HIV were less likely to experience an NFOD (IRR: 0.83; 95%CI: 0.80, 0.86) in comparison to males living without HIV.
Conclusion: Our analyses demonstrate that among PLWH, females experience a higher incidence of NFOD events. This sex disparity is important to explore further, given the potential for adverse health outcomes after NFODs. These findings have implications by suggesting the need for policies and programs oriented toward women to mitigate overdoses, especially those living with HIV.
**Conclusion:** TGW from TransCITAR presented poor health outcomes: high prevalence of HIV/syphilis, high proportion with incomplete/no HBV vaccine and high levels of depression and violence. A comprehensive approach to care and addressing social determinants of health is pivotal to reduce HIV burden in this population.

| Table 1. Baseline factors associated with HIV diagnosis. |
|---|---|---|---|---|---|
| Characteristics | N=974 | N=258 | N=616 | N=974 | P-VALUE |
| Age (median, IQR) | 42 (35-50) | 42 (35-50) | 42 (35-50) | 0.320 |
| Sex (Female) | 372 (38.2%) | 101 (39.3%) | 271 (44.2%) | 0.149 |
| Race (White) | 713 (73.0%) | 196 (75.9%) | 517 (83.9%) | 0.001 |
| Education (Less than complete high school) | 1219 (74.1%) | 312 (74.3%) | 907 (74.1%) | 0.858 |
| Unemployment (Yes) | 272 (28.0%) | 71 (27.7%) | 191 (24.5%) | 0.265 |
| sew work experience (yes) | 1219 (74.1%) | 312 (74.3%) | 907 (74.1%) | 0.858 |
| Depression (yes) | 144 (14.9%) | 38 (14.9%) | 106 (13.7%) | 0.660 |
| Current tobacco smoking (yes) | 173 (17.8%) | 43 (16.8%) | 130 (16.9%) | 0.593 |
| Heart disease (yes) | 460 (46.8%) | 107 (41.9%) | 353 (45.0%) | 0.201 |
| Drug abuse (yes) | 122 (12.6%) | 33 (12.9%) | 89 (11.4%) | 0.604 |
| Cocaine use last year (yes) | 137 (13.9%) | 32 (12.5%) | 105 (13.0%) | 0.613 |
| Sexual violence, lifetime (yes) | 862 (88.3%) | 225 (87.4%) | 637 (81.2%) | 0.033 |
| Physical violence, lifetime (yes) | 204 (20.9%) | 54 (21.0%) | 149 (19.2%) | 0.636 |
| Psychological IPV, lifetime (yes) | 207 (21.0%) | 55 (21.3%) | 152 (19.4%) | 0.636 |

**Conclusion:** IPV and important health outcomes and behaviors using linear and logistic regression and provide appropriate healthcare intervention. Assess both psychological and physical IPV among PWH as part of routine care and determine the extent of problem use depending on the type of IPV, suggesting the need to assess both psychological and physical IPV among PWH as part of routine care and provide appropriate healthcare intervention.

| Table 1. Association of IPV with outcomes, logistic and linear regression, and provide appropriate healthcare intervention. |
|---|---|---|---|---|---|---|---|
| IPV (yes) | Log odds | 95% CI | P-value | β-adjusted | 95% CI | P-value |
| Physical violence | 1.31 | 0.80-2.10 | 0.312 | -0.13 | -0.26-0.003 | 0.068 |
| Psychological violence | 1.66 | 1.02-2.70 | 0.043 | 0.16 | 0.02-0.30 | 0.022 |
| Psychological violence | 1.29 | 0.86-2.00 | 0.211 | 0.09 | -0.03-0.20 | 0.119 |

**Conclusion:** IPV and important health outcomes and behaviors using linear and logistic regression and provide appropriate healthcare intervention. Assess both psychological and physical IPV among PWH as part of routine care and determine the extent of problem use depending on the type of IPV, suggesting the need to assess both psychological and physical IPV among PWH as part of routine care and provide appropriate healthcare intervention.
Methods: Using cross-sectional data from a prospective cohort study in Kenya, we identified the prevalence and correlates of violence among sexual and injecting partners of PWID living with HIV, whom we contacted through assisted partner services. Violence is defined as any physical harm (hit, slap, kick, physically hurt), threats with a weapon or mentally, or forced sexual acts inflicted on a person by anyone in the past year. We used a Chi-squared test and a two-sided Fisher's exact test to identify the socio-demographic characteristics associated with violence. Using the Woolf test for homogeneity, we conducted a stratified analysis to test effect modification by gender and HIV status.

Results: Among 1302 partners, 1439 (44%) experienced violence within the past year. Physical violence was the most common form of violence experienced (35%; 95% confidence interval [95% CI] 33.3%, 36.5%), followed by being threatened (23%; 95% CI 21.5%, 24.9%), and sexual violence (7%; 95% CI 6.2%, 7.9%). Being male (Relative Risk [RR] = 1.22; 95% CI 1.11, 1.33; p < 0.001), living in coastal Kenya (RR = 1.53; 95% CI 1.41, 1.66; p < 0.001), having multiple sexual partners (RR = 1.39; 95% CI 1.22, 1.6; p < 0.001), being divorced/separated/widowed (vs. single) (RR = 1.24; 95% CI 1.13, 1.37; p < 0.001), not having stable housing (RR = 1.14; 95% CI 1.03, 1.27; p = 0.019), being both a sexual and injecting partner of a PWID (vs. injecting partner only) (RR = 1.18; 95% CI 1.06, 1.32; p = 0.005), being an active injection drug user not on methadone (vs. on methadone) (RR = 1.53; 95% CI 1.04, 2.25; p = 0.018), and, for men, having had sex with men (RR = 1.36; 95% CI 1.21, 1.54; p < 0.001) were associated with experiencing violence. The stratified analysis identified that gender was an effect modifier while HIV status was not.

Conclusion: Study results highlight PWID partners at increased risk for experiencing violence, which may help to formulate and tailor effective public health interventions and policy recommendations for increasing HIV-related services among key populations in Kenya.

Table: Stratified analysis by Gender on the association between some partner characteristics and experiencing violence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of individuals</th>
<th>Odds Ratio (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>194</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>0.96 (0.89-1.05)</td>
<td>1.01 (0.99-1.06)</td>
</tr>
<tr>
<td><strong>Partner type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both sex &amp; inject</td>
<td>1.16 (1.06-1.22)</td>
<td>1.02 (0.89-1.17)</td>
</tr>
<tr>
<td><strong>Partner type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injecting</td>
<td>1.00 (1.00-1.00)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td><strong>Employment status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informal</td>
<td>0.96 (0.81-1.13)</td>
<td>0.95 (0.80-1.11)</td>
</tr>
<tr>
<td>Formal</td>
<td>1.50 (1.41-1.60)</td>
<td>1.59 (1.46-1.77)</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nairobi</td>
<td>1.00 (1.00-1.00)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>Coast</td>
<td>1.35 (1.23-1.48)</td>
<td>2.04 (1.75-2.30)</td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal</td>
<td>1.00 (1.00-1.00)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>Informal</td>
<td>0.99 (0.95-1.03)</td>
<td>0.95 (0.90-1.01)</td>
</tr>
</tbody>
</table>

Among MSM, 66% had recent unprotected sex with a man at their first test. Among PWID, 63% were actively injecting and 39% recently shared injecting paraphernalia. There were a total of 1039 incident HIV infections. The overall incidence rate for MSM and PWID were 1.9/100 PY (95% CI: 1.7-2.2) and 4.1 (3.9-4.4), respectively. Among MSM sites, incidence ranged from 0.4 to 3.5 and in PWID sites from 0.6 to 19.1. Among MSM, incidence increased significantly each year (0.7 in 2015 to 2.9 in 2022). Among PWID, incidence increased significantly until a peak in 2020 and then significantly declined (1.8 in 2015, 5.6 in 2020, 3.8 in 2022). Across populations/cities these trends were generally similar though the magnitude of incidence was highly variable (Fig 1).

Conclusion: While there was substantial geographic variability, nearly all experienced high incidence (~2-100PY) despite being currently engaged in a community-based clinic where preventive services are free. This highlights the need to focus on KP in LMICs when considering novel strategies such as long acting PrEP to curtail incidence.

HIV incidence among MSM and PWID in Integrated Care Centre clients with a 2-month moving average, 2015-2022 in India
offered an HIV test during a visit. More than half delayed healthcare due to injection drug use stigma (55%) and discrimination (54%) when accessing healthcare. With respect to 95-95-95 targets, 80% of PWID living with HIV were previously diagnosed, of whom 92% were on antiretroviral treatment, of whom 71% were virally suppressed.

Conclusion: San Francisco is not on course to get to zero HIV infections for PWID, progress toward 95-95-95 targets is slow, and stigma remains high. Our data corroborate citywide HIV case reporting suggesting recent increases in new HIV infections among PWID despite decreases in the epidemic overall. Our data point to a need for HIV testing in healthcare missed opportunities for early diagnosis, and challenges for retention in care and viral suppression. PWID-sensitive and focused programs are needed to increase HIV testing overall, sustain retention in care, and address stigma if San Francisco is to end the epidemic for all.

865 RACIAL DISPARITIES IN HIV PRE-EXPOSURE PROPHYLAXIS-RELATED OUTCOMES IN MALE VETERANS
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Background: Despite its known efficacy, human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) has had limited uptake in United States (US) Veterans at high risk for HIV acquisition. We hypothesized that Black US Veterans of minoritized sexual orientation (MSO) receive PrEP at comparable rates relative to other racial categories. However, we also hypothesized that higher rates of HIV acquisition would be associated with Black race, regardless of PrEP receipt.

Methods: A retrospective analysis was conducted for the period of July 12, 2012 through July 1, 2019, using a validated natural language processing system to derive a cohort of Veterans documented as MSO. HIV diagnosis was defined as the first appearance of the diagnosis in the electronic health record (EHR) as identified by an International Classification of Disease code. PrEP recipients were individuals with two or more pharmacy fills of emtricitabine/tenofovir disoproxil fumarate. Race was defined by structured categories available in the Veterans Affairs EHR. Chi-square tests evaluated the association between race and HIV acquisition stratified by PrEP use (α=0.05).

Results: A cohort of 67,299 HIV-negative male Veterans with MSO documentation was identified. In total, 2,375 received PrEP and 64,924 did not. Of the 11,161 Black Veterans, 459 (4.1%) received PrEP, while 1621 (3.2%) of the 50,336 White Veterans and 295 (3.5%) of the 5,782 Other Veterans received PrEP. For those who received PrEP, 10.5% of Black Veterans, 8.1% of White Veterans, and 8.8% of Other Veterans acquired HIV. The association between HIV acquisition and race was not statistically significant in PrEP recipients. For those who did not receive PrEP, 9.8% of Black Veterans, 2.8% of White Veterans, and 4.8% of Other Veterans acquired HIV. The association between HIV acquisition and race was statistically significant in PrEP non-recipients.

Conclusion: Rates of PrEP receipt in Veterans with MSO documentation were comparable across racial groups. HIV acquisition was lower in White and Other Veterans who did not receive PrEP, suggesting that patients and providers assessed their risk as lower than those who were offered and accepted PrEP. The lack of full protection in those receiving PrEP reinforces the real-world occurrences of gaps in adherence and persistence. For Black Veterans, rates of HIV acquisition were comparable for those who did and did not receive PrEP. These findings suggest a higher baseline risk for HIV acquisition independent of PrEP receipt.

HIV Acquisition by Racial Category for Veterans Who Did and Did Not Receive PrEP

<table>
<thead>
<tr>
<th>Race</th>
<th>PrEP Recipients</th>
<th>Other Veterans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total N = 64,924</td>
<td>Black N = 10,172</td>
</tr>
<tr>
<td>HIV acquisition</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Total N = 62,226</td>
<td>HIV N = 988</td>
</tr>
<tr>
<td></td>
<td>HIV N = 11,161</td>
<td>HIV N = 1,122</td>
</tr>
<tr>
<td></td>
<td>HIV N = 2,375</td>
<td>HIV N = 230</td>
</tr>
</tbody>
</table>

866 BEHAVIOR CHANGE AMONG HIV-NEGATIVE MEN WHO HAVE SEX WITH MEN NOT USING PrEP IN THE US
Steven M. Goodreau1, Michael P. Barry2, Deven T. Hamilton3, Travis H. Sanchez1, Kevin Delaney4
1University of Washington, Seattle, WA, USA, 2Emory University, Atlanta, GA, USA, 3Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: HIV incidence is falling among US gay, bisexual and other men who have sex with men (MSM) as a result of HIV pre-exposure prophylaxis (PrEP) and HIV treatment as prevention; however, >20,000 diagnoses still occur among MSM each year. Considerable research has documented behavior change among MSM on PrEP. We know of no studies to consider such change among HIV-negative US MSM not using PrEP, even though they still comprise the majority of MSM and are presumably at greatest seroconversion risk. We hypothesized that such men may see increasing rates of condomless anal sex (CAS) either with partners believed to be on PrEP or suppressed (indirect protection), or more generally given changing norms around condom use. We also predicted that increases would be largest for young MSM and vary by race/ethnicity.

Methods: We used serial cross-sectional data from the American Men’s Internet Survey (2014-2019), limited to the subsample enrolled in 2 consecutive years and reporting being HIV-negative and not using PrEP both years (HNNP-2, n=2,421). We estimated the proportion reporting CAS each year, and used one-sided McNemar tests to identify significant increases. We then disaggregated by partner status (unknown, positive, negative). Among HNNP-2 respondents reporting 2+ CAS partners each year, we used Wilcoxon signed rank tests to identify significant increases in number of CAS partners in year 2.

Results: Overall, CAS increased by 2 percentage points (pp) from year 1 to year 2 (68-70%); increases were largest among younger (age 15-24, 5pp) and Hispanic/Latino (Latino) (5pp) respondents; especially among younger Latinos (1pp, from 68% to 87%). Increases were concentrated among those with perceived HIV-negative partners. Additional predictors included lower education and income, and residence on metropolitan fringe. Younger participants also had significant increases in the number of CAS partners year-over-year.

Conclusion: Among HIV-negative MSM not on PrEP, younger and Latino males are experiencing sizeable increases in CAS. Patterns suggest this is unlikely to represent effective reliance on indirect protection (partners on PrEP or suppressed). This signals a need to increase access to HIV prevention messaging specifically for these populations. Although efforts often focus on getting MSM on PrEP, it is also key to enhance counseling about risk among those who are unable or unwilling to initiate PrEP, as other prevention measures may be getting less visible in these communities.

867 REASONS FOR MIGRATION AND ASSOCIATIONS WITH HIV RISK AMONG SEXUAL MINORITY MEN
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Background: The U.S. population of immigrants has grown rapidly over the past two decades. Knowledge about reasons for migration and their association with HIV-related behaviors is limited. We explored patterns in reasons for migrating to the U.S. among cisgender gay, bisexual, and other sexual minority
men (SMM) and determined their associations with HIV risk and prevention behaviors.

Methods: Data were from the 2018-2020 cycles of the American Men’s Internet Survey, an annual online survey of SM in the U.S. who report having sex with other men in the past year. We limited analyses to participants born outside the U.S. who reported reason(s) for migration and reported HIV-negative/unknown status. Participants selected from a list of reasons and could write in a reason. Latent Class Analysis (LCA) was performed to identify patterns in the reasons for migration. We used multivariate logistic regression controlling for demographic characteristics to assess class associations with the following in the past 12 months: condomless anal sex (CAS), illicit drug use, marijuana use, HIV testing, and PrEP use.

Results: Among 1,657 SMM, 21% were from Mexico, 18% from Europe, and 13% from South America; 29% spoke a primary language other than English. LCA identified 6 distinct patterns in reasons for migration: 1) Family and friends (14%); 2) Financial (17%); 3) Personal freedom related to being gay (10%); 4) Pursuit of opportunities while living openly as SMM (12%); 5) Educational purposes (18%); 6) Not my decision (29%). While HIV testing (range = 57.6-65.4%) and PrEP use (55.6-21.4%) did not vary by class (p > .05 for all), CAS and illicit drug use were significantly different (Table). SMM who migrated to pursue opportunities while living openly and whose reasons were not their decision had greater odds of CAS than SMM who migrated for educational purposes (adjusted odds ratio (aOR) = 1.72, 95% confidence interval (95%CI): 1.15–2.59; 1.57, 1.13–2.19, respectively). SMM who migrated because of personal freedom related to being gay and for educational purposes had lower odds of illicit drug use than SMM who migrated due to family and friends (aOR, 95%CI: .57, .35–.93; .67, 45–1.00, respectively).

Conclusion: Reasons for migrating to the U.S. among SMM were associated with behaviors that can increase HIV risk. Push and pull factors related to migration matter when considering the HIV prevention needs of immigrant SMM.

Latent Class Analysis (LCA) Model: Comparisons of HIV Risk and Prevention Behaviors By Reasons for Migration Latent Classes

Table: Latent Class Analysis (LCA) Model: Comparisons of HIV Risk and Prevention Behaviors By Reasons for Migration Latent Classes

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</tr>
</thead>
<tbody>
<tr>
<td>CAS</td>
<td>1.00 (1.00–1.00)</td>
<td>1.39 (1.07–1.81)</td>
<td>1.39 (1.07–1.81)</td>
<td>1.40 (1.06–1.85)</td>
<td>1.40 (1.06–1.85)</td>
<td>1.40 (1.06–1.85)</td>
</tr>
<tr>
<td>IVDU</td>
<td>1.00 (1.00–1.00)</td>
<td>1.19 (0.87–1.64)</td>
<td>1.19 (0.87–1.64)</td>
<td>1.19 (0.87–1.64)</td>
<td>1.19 (0.87–1.64)</td>
<td>1.19 (0.87–1.64)</td>
</tr>
</tbody>
</table>

868 SEXUAL RISK AND ROLE OF LOW-LEVEL VIREMIA TO HIV TRANSMISSION IN SUB-SAHARAN AFRICA

Olanrewaju Edun1, Lucy Okell1, Helen M. Chum1, Kyle Milligan3, Emilio Dirlikov4, Ray W. Shiraishi2, Jeffrey W. Eaton1

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Imperial College London, London, United Kingdom, R2
Centers for Disease Control and Prevention, Atlanta, GA, USA, R3
Paratan, Nairobi, Kenya, USA, R4
Centers for Disease Control and Prevention, Kampala, Uganda

Background: Sexual HIV transmission can occur when people with HIV (PHIV) have detectable viremia and unprotected sexual contact with susceptible partners. Low-level viremia (LLV) 51–999 viral copies/mL among PHIV on antiretroviral therapy (ART) can lead to HIV transmission even as ART coverage rises. We assessed differences in sexual risk behaviours and distribution of viral load (VL) at stages of the HIV cascade, including LLV, and modelled the contribution to transmission.

Methods: We analysed data for adults (≥15 years) from Population-based HIV Impact Assessment (PHIA) surveys conducted in 13 sub-Saharan African countries during 2015–2019, incorporating survey weights. We used log-binomial regression to estimate adjusted prevalence ratios (aPR) of high-risk behaviours (multiple partners and condomless sex in past year) for five cascade categories: undiagnosed, untreated, on ART but non-suppressed (≥1000 copies/mL), on ART with LLV (51–999 copies/mL), and suppressed (≤50 copies/mL). We calculated the mean log10-VL and transmission rate by cascade group and sex. A transmission equation incorporating the proportion of individuals, prevalence ratio of high HIV risk behaviour, and transmission rate was used to quantify the proportion of transmission from each subgroup.

Results: Compared to suppressed PHIV, reported high-risk behaviour was more likely among undiagnosed PHIV (aPR for women: 1.25, 95%CI: 1.06–1.48; aPR for men: 1.59, 95%CI: 1.32–1.93) and men diagnosed but untreated (aPR: 2.05, 95%CI: 1.32–1.93). There was no significant association between LLV and high-risk behaviour. The mean log10-VL and estimated transmission rate was similar between those undiagnosed, diagnosed but untreated and those on ART but non-suppressed, but lowest among individuals with LLV. Undiagnosed and diagnosed but untreated PHIV contributed most to estimated transmission (73–92%), with low (<1%) estimated transmission from those with LLV.

Estimated transmission from individuals on ART but non-suppressed increased over time as awareness of status and ART coverage increased.

Conclusion: Undiagnosed and diagnosed but untreated PHIV account for the majority of transmissions in sub-Saharan Africa, highlighting a need for increased access to HIV testing and ART linkage services. Transmission from PHIV with LLV is low. In countries with high ART coverage, non-suppressed individuals on ART account for an increasing share of transmission, underscored by the importance of maintaining high viral suppression levels.

Figure. Pooled estimated proportion of HIV across the 13 surveyed countries and estimated transmission proportion attributable to PHIV subgroups, 2010 to 2020. Data on proportion of PHIV outside survey years were obtained from UNAIDS country estimates.

869 REDUCTION OF HIV INCIDENCE AFTER ELIMINATION OF LYMPHATIC FILARIASIS

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Background: A general population survey from 2007 to 2011 in Southwest Tanzania previously revealed a 2.3-fold increased HIV incidence among individuals infected with Wuchereria bancrofti (WB), the parasite causing lymphatic filariasis. In 2007, the prevalence of filarial and HIV infections were 36.4% (385/1058) and 13.8% (162/1173), respectively, for individuals aged 14 to 65. A government program distributed the antifilarial drugs ivermectin and albendazole annually from 2009 to 2015. This intervention was evaluated in 2019 and a WB prevalence of 1.7% (22/1299) was found in 14 to 65 year olds. The impact of the quasi-elimination of WB on the HIV incidence is evaluated here.

Methods: Individuals who participated in the survey from 2007 to 2011 were re-visited in 2019 and screened for WB with a strip test detecting circulating filarial antigen and HIV using the government test algorithm.

Results: 1299 individuals from the first survey who were 14–65 years old at the current visit (2019) were able to participate in the screening. 116 (8.9%) of them had been HIV-infected at the end of the previous study in 2011. Among the 1183 participants without prior HIV, 57 new HIV infections occurred between 2011 and 2019. Among these 1183 participants, 372 (31%) had been previously
infected with *W. bancrofti* but were now cured. Between 2011 and 2019, 21 new HIV infections were seen in 2747 PY, HIV incidence 0.76/100 person years (PY). This is compared to 17 HIV infections in 850 PY (HIV incidence 1.9/100 PY) between 2007 and 2011, during which these individuals had been infected with *W. bancrofti*. The incidence rate ratio (IRR) of 0.39 was shown to be significant, p=0.0036 by using a Poisson regression and adjusting for age and gender. Among the continuously filarial-uninfected 673 study participants, 38 HIV new infections occurred between 2011 and 2019 in 5915 PY (HIV incidence 0.64/100PY), as compared to 9 incident HIV infections between 2007 and 2011 in 1250 PY (HIV incidence 0.72/100PY, IRR of 0.92, p=0.815).

**Conclusion:** The increased HIV incidence of 1.9/100 PY seen in filarial-infected individuals from 2007 to 2011 dropped significantly to 0.76/100 PY between the 2011 and 2019 surveys, after years of effective antifilarial treatment. At the same time, the HIV incidence among the filarial-uninfected group declined only slightly from 0.7/100 PY in 2007 to 2011 to 0.64/100 PY between 2011 and 2019. These findings highlight that elimination of NTDs can lower the HIV incidence and help reduce the spread of HIV.

870 **TRENDS IN MORTALITY IN PEOPLE LIVING WITH HIV IN AN INTERNATIONAL COHORT (RESPOND)**

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**Background:** Mortality rates in people living with HIV (PLWH) have declined in recent years due to effective antiretroviral treatment (ART). Aging, coinfections, and comorbidities among PLWH may drive further changes in mortality.

**Methods:** Mortality due to specific causes classified by the Coding Causes of Death in HIV (CoDe) methodology were investigated between 2012–19 among PLWH from the RESPOND cohort consortium. Age-standardized mortality rates were calculated over 2-year periods and age-adjusted Poisson models were used to compare mortality between periods. We also investigated all-cause mortality with multivariable Cox regression.

**Results:** Among 33642 PLWH with 170084 person-years of follow-up (PYFU) (median PYFU 4.9; IQR 3.1–8.0), 1702 (5.1%) died. Crude all-cause mortality per 1000 PYFU decreased from 13.0 (95% CI 11.8–14.4) in 2012–13 to 8.4 (95% CI 7.7–9.3) in 2018–19. Median age increased from 2012–13 to 2018–19 (44 years IQR 36—51) to 2018–19 (49 IQR 40—56) as did median age at death from 52 IQR 45—62 to 56 IQR 48—65. The leading cause of death across the entire period was non-AIDS defining malignancy (NADM): 2.18 per 1000 PYFU (95% CI 1.96—2.41). Age-adjusted Poisson regression showed statistically significant decreases in mortality from 2012–13 to 2018–19 for deaths due to NADM, AIDS, cardiovascular disease (CVD), and liver disease (figure). The proportion of deaths due to AIDS (13.1% to 7.9%) and liver disease (11.0% to 4.9%) declined from 2012–13 to 2018–19, and increased due to NADM (16.5% to 22.5%) and CVD (7.6% to 9.9%), but with concomitant increase in deaths due to unknown/missing causes (18.8% to 30.6%). In multivariable analysis, the strongest predictor of all-cause mortality was current CD4 ≤200 cells/µl (HR: 3.42 (95%CI 1.63, 7.18) at ≤35 years to 0.92 (95%CI 0.56, 1.53) at ≥70 years. Risk of NADM mortality was higher in participants that acquired infection with modifiable risk factors, indicating areas for improvement.

Conclusion: In the RESPOND cohort, the leading cause of death was NADM. Age-adjusted mortality rates from AIDS, NADM, CVD, and liver-related causes declined, especially 2012—13 to 2016—17; the role of other contributing factors will be explored further. All-cause mortality was strongly associated with modifiable risk factors, indicating areas for improvement.

871 **NON-AIDS DEFINING MALIGNANCY MORTALITY IN PEOPLE LIVING WITH HIV**

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**Background:** Our aim was to evaluate rate and prognostic factors of mortality due to non-AIDS defining malignancies (NADM) among persons living with HIV (PLWH) from the cohort of the Spanish AIDS Research Network (CoRIS) during 2004–2020.

**Methods:** We included antiretroviral-naive individuals aged ≥ 20 years at enrolment, recruited during 2004–2020. NADM deaths were all deaths due to cancer, except those due to AIDS defining malignancies such as Kaposi sarcoma, certain types of non-Hodgkin lymphomas and cervical cancer. We estimated mortality rates and standardised mortality ratios (SMRs) using NADM mortality rates from the Spanish general population. We applied cause-specific Cox proportional hazard models, accounting for competing risk, and age as time-scale to identify prognostic factors for NADM mortality.

**Results:** Of the 17,329 study participants, 85% were men and median age was 35 years. The overall mortality rate was 1.53 (95% confidence interval: 1.32, 1.79) per 1,000 person-years (PY), 76% higher as that in the general population (SMR: 1.76, 95% CI 1.51, 2.06). The highest mortality rates were found for lung (0.56 per 1,000 PY; 95% CI 0.44, 0.73) and liver cancer (0.18 per 1,000 PY; 95% CI 0.12, 0.28). Mortality rates increased with age, whereas SMRs decreased from 3.42 (95% CI 1.63, 7.18) at ≤35 years to 0.92 (95% CI 0.56, 1.53) at ≥70 years. Risk of NADM mortality was higher in participants that acquired infection through heterosexual contact (Hazard Ratio: 1.48; 95% CI 1.07, 2.03) and injection drug use (HR: 1.54 (0.87, 2.76), compared to men who have sex with men. Time-varying prognostic factors for NADM mortality were: active smoking (HR: 2.23; 95%CI 0.96, 5.18), presence of hepatitis C virus antibodies (HR: 1.74; 95%CI 1.09, 2.07) or hepatitis B surface antigen (HR: 2.02; 95%CI 1.12, 3.66) and decreasing CD4 count (HR: 1.19; 95%CI 1.56, 13.20 for CD4 < 200 cells/µl; HR: 4.10; 95%CI 2.57, 6.55 for CD4 200–349 cells/µl;HR: 2.50; 95%CI 1.64, 3.80 for CD4 350–499 cells/µl compared to CD4<200 cells/µl).

**Conclusion:** Mortality due to NADM in PLWH is higher than in the general population, mainly at younger ages. Smoking, viral hepatitis coinfections and immunosuppression independently increased risk of death due to NADM.
PROPORTION OF DEATHS OCCURRING OUTSIDE OF HEALTH FACILITIES ATTRIBUTABLE TO HIV/AIDS

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Background: Reliable and accurate mortality and cause-of-death data are lacking for deaths occurring outside health facilities in Malawi, including HIV/AIDS-related deaths. According to Malaria Vaccine Implementation Program (MVIP) data, over 70% of deaths in Malawi occur in the community. Malawi is piloting cause-of-death reporting in community using the 2016 World Health Organization (WHO) verbal autopsy (VA) tool. We used data from this pilot to estimate proportions of deaths occurring in the community that are due to HIV/AIDS in Malawi.

Methods: The pilot implementation is taking place in K cholira and Nkanda clusters in Mchinji district where a community death registration system is already established and has an HIV prevalence of 5.4%. VA interviews are conducted with consenting “primary caregivers” of the deceased by trained mortality surveillance assistants using the 2016 WHO VA electronic questionnaire on OpenDataKit. Primary caregivers are persons who were with the deceased in the period leading to the death. Using InterVA5 software, we analyzed VA data collected between January and August 2022. We compared the VA data with health facility mortality data collected within the same period in the same district.

Results: 354 deaths were recorded in the two clusters, of which 54% (190/354) occurred in the community. Cause of death was assigned to 91% ([172/190]) of community deaths vs 52% ([86/164]) of health facility deaths. The median age of the deceased were 69 years (95% CI: 58-73) for community deaths and 44 years (95% CI: 24-57) for health facility deaths. The proportion of females was 45% ([77/172]; 95% CI: 38%-52%) among the community deaths and 44% ([138/304]; 95% CI: 34%-55%) among health facility deaths. For community deaths, HIV/AIDS was the second leading probable cause of death (17% [29/172]) 95% CI: 12%-23%; after unspecified-cardiac diseases (17% [30/171]; 95% CI: 13%-24%). For health facility deaths, HIV/AIDS was the third leading probable cause of death (8% [7/86] 95% CI: 4%-16%).

Conclusion: Malawi is piloting the 2016 WHO VA tool with almost all deaths sampled from the targeted communities successfully assigned a cause of death. We found a higher proportion of deaths attributed to HIV/AIDS among community deaths than among health facility deaths. Scale up of VA may improve the accuracy of national cause-of-death and HIV/AIDS-related mortality estimates in Malawi.

MEASURING EXCESS MORTALITY ASSOCIATED WITH HIV: ESTIMATES FROM THE PHIA PROJECT

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Background: Assessing national HIV epidemics requires accurate data on new HIV infections and HIV-related deaths. Mortality estimates are challenging in settings with incomplete vital statistics. We used unique survey data on household-level mortality and HIV status to estimate excess mortality associated with HIV epidemics. We compared mortality between individuals in households (HH) with and without people living with HIV (PLWH) and explored how a diagnosis of tuberculosis (TB) may modify mortality estimates.

Methods: We used publicly available data from 6 Population-based HIV Impact Assessment (PHIA) surveys conducted in Cameroon, Cote d’Ivoire, Ethiopia, Malawi, Tanzania, and Zimbabwe between 2015-2018. Heads of HH of randomly selected HH were asked to list all usual HH members and whether any usual HH members died in the 3 years before the survey. For each death reported, they indicated sex of the deceased, age at death and date of death. All consenting HH members answered questions about their TB status and provided blood for rapid HIV testing. We calculated the number of reported deaths per 1,000 for the 3-year period before the survey, and measured mortality differences associated with the presence of documented HIV and/or self-reported TB among HH members. Analyses included all usual HH members. Multi-country weighted deaths per 1,000 were calculated.

Results: The number of HH in each survey ranged from 8,983 in Cote d’Ivoire to 14,811 in Tanzania. The percent of HHs reporting any deaths in the prior 3 years ranged from 3.7%-12.9%. The estimated crude number of deaths in the prior 3 years ranged from 11.4 in Ethiopia to 32.7 per 1000 in Cameroon. In multi-country analyses, the number of weighted deaths per 1,000 were 29.3 (95% Confidence Interval [CI]: 27.2-31.0) versus 20.9 (95% CI: 20.2-21.6) for individuals in HH with and without PLWH; 26.4 (95% CI: 23.3-29.2) versus 21.7 (95% CI: 21.0-22.3) for those in HH with and without TB case(s); and 28.0 (95% CI: 26.3-29.6) versus 20.8 (95% CI: 20.1-21.5) for those in HH with HIV and/or TB versus without HIV or TB (Figure).

Conclusion: In this first description of mortality using PHIA data from 6 nationally representative surveys, HH with residents who had HIV and/or TB had significantly higher numbers of deaths compared to HHs with residents who had neither. These findings suggest a new approach, which captures indirect effects of HIV on mortality of HH members, to tracking progress towards zero AIDS-related deaths.

Multi-country weighted average deaths per 1,000 population over 3 years before PHIA survey by household comorbidity, Cameroon, Cote d’Ivoire, Ethiopia, Malawi, Tanzania, Zimbabwe, 2015-2018

874 ONE-YEAR MORTALITY OF PEOPLE WITH HIV AFTER INTENSIVE CARE UNIT ADMISSION

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Background: People with HIV (PWH) continue to experience opportunistic infections and critical illness, increasing their risk of, and mortality after, intensive care unit (ICU) admission. The literature-reported data on risk of death in and after discharge from ICU are limited. We describe trends in 1-year mortality among adult PWH (>18 years) admitted to ICU from 2000-2019 in an HIV-referral centre.

Methods: One-year mortality was calculated from index ICU admission to date of death; follow-up was right-censored at day 365 if the person was known to remain alive at 1 year, or day 7 after ICU discharge if known to be alive at hospital discharge but lost to follow-up. Between-group differences in characteristics at admission by calendar year (2000-3, 2004-7, 2008-11, 2012-15 and 2016-19) were explored using Kruskal Wallis/Cochrane Armitage tests, and 1-year mortality using Kaplan-Meier/log-rank tests. Cox regression described associations with calendar year (as a continuous covariate) before and after adjustment for characteristics at ICU admission: age, sex, Acute Physiology and Chronic Health Evaluation II (APACHE II), CD4+ T-cell count, and recent HIV diagnosis (within 3 months of ICU admission).

Results: 221 PWH were admitted to ICU (72% male, median [interquartile range [IQR]] age 45 [38-53] years) of whom 108 had died within 1-year (median survival: 349 days, cumulative 1-year survival: 50%). Those admitted in later years had a lower median admission APACHE II (29, 25, 17, 14, 13 respectively, p<0.001) and were older (medians of 40, 44, 44, 46, 49 years, respectively, p=0.03), with higher median CD4 + T-cell count (98, 52, 169, 212, 128 cells/μm³, p=0.002), lower percentage with advanced HIV (CD4+ T-cell count <200 cells/μm³ and/or AIDS at admission to ICU; 95, 77, 66, 50, 66%, p=0.01) and greater percentage with HIV-RNA <50 copies/ml (17, 34, 59, 46, 33%, p=0.02). Cumulative survival increased in later years (Figure, p=0.001, log-rank test), with an 11% reduction in the hazard of 1-year mortality per later year (hazard
Conclusion: Though groups like the World Health Organization rely on weights derived from Sub-Saharan African populations to correct for mortality misclassification in Latin America, our results demonstrate that local registry linkage can be successfully used to reduce measurement error in survivorship for these populations.

Figure. Kaplan-Meier curves for unadjusted survival probabilities after ART initiation, pre- and post-linkage, by site.
877 IMPACT OF REDLINING ON TIME TO VIRAL SUPPRESSION AMONG PERSONS DIAGNOSED WITH HIV

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**Background:** Developed in the 1930s by the US government, Home Owners’ Loan Corporation (HOLC) Security Maps classified residential neighborhoods with populations of 40000 or more into grades based on the alleged investment risk of resident borrowers. Grades ranged from A (mapped as green areas considered “Best”) to D (or red areas that were poorer and predominantly Black neighborhoods considered “Hazardous”), the latter colloquially referred to as “Redlined.” The ongoing impact of redlining on chronic diseases such as obesity and diabetes has been described previously. Similar associations with HIV outcomes could help direct local Ending the HIV epidemic (EHE) Initiatives. We seek to assess the association of structural racism through redlining policies on current time to viral suppression (VS) among people newly diagnosed with HIV in two cities in Louisiana, a state with one of the highest HIV incidence rates in the country.

**Methods:** City boundaries were defined by 2020 U.S. Census Bureau Zip Code Tabulation Areas and spatially joined with HOLC-graded neighborhoods. Redlined neighborhood residences of new HIV diagnoses from 2011-2019 were determined using Louisiana HIV surveillance data. Unadjusted and adjusted estimates for time to VS by redline status were calculated using Kaplan-Meier survival analysis.

**Results:** Of the 3227 PWH analyzed, 929 (28.8%) lived in a redlined neighborhood, 606 (18.8%) lived in A, B or C HOLC neighborhoods, and 1692 (52.4%) lived within the city but not in a HOLC red area. In redlined areas, 74.1% of PWH were Black as compared to 63.5% in A, B, and C grade areas, and 78.3% in non-graeded areas. The adjusted median time to VS among PWH in redlined areas was 262.0 days (95% CI: 219.0-294.0), compared to 195.0 days (95% CI: 182.0-207.0) among PWH in non-redlined areas. PWH in redlined areas was 0.88 (95% CI: 0.18-0.95) times as likely to achieve VS as PWH in non-redlined areas.

**Conclusion:** Generational inequities and structural racism continue to impact present-day health outcomes. Time to VS was significantly longer for PWH in redlined vs non-redlined communities. Our analysis quantifies the importance of the physical environment on health outcomes in Louisiana, and these findings can inform local EHE strategies and beyond. Time to VS from HIV diagnosis by residence within Redlined areas, Shreveport and New Orleans, 2011-2019

878 MONITORING CARE AND VIRAL SUPPRESSION AFTER HIV DIAGNOSIS IN UNITED STATES: 2017-2020

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**Background:** The Ending the HIV Epidemic in the U.S. (EHE) initiative aims to significantly reduce HIV transmission in the United States by 2030. Key EHE strategies include rapid linkage to care and prompt delivery of antiretroviral treatment to people with HIV after diagnosis. We evaluated a jurisdictional proposal to use early HIV viral suppression as an outcome indicator for monitoring national progress in HIV care.

**Methods:** Data reported to the National HIV Surveillance System through December 2021 were used to determine linkage to HIV care (= 1 CD4 or viral load (VL) tests within 1 month of diagnosis) and early viral suppression (VL < 200 within 3 months of diagnosis) among persons aged ≥ 13 years with HIV diagnosed during 2017 (EHE baseline) through 2020. Data were analyzed by gender identity, age, race/ethnicity, HIV transmission category, population density of area of residence, and area of residence at diagnosis (not shown in table) for persons residing in 41 jurisdictions (40 states and the District of Columbia) with complete reporting of CD4 and VL results. Estimated annual percentage change (EAPC) and 95% CIs were calculated to assess trends, which were considered statistically significant if the EAPC confidence intervals (CIs) excluded 0.

**Results:** Overall, the percentage of persons linked to HIV medical care within 1 month of diagnosis increased 2.0% (CI: 1.7–2.2) per year from 2017-2020 (Figure 1). By subpopulation, increases ranged from 0.9% (CI: 0.5–1.4) among White persons to 2.8% (CI: 1.2–4.3) among Asian persons per year. Linkage to care increased in areas of all population densities and in 13 of the 41 jurisdictions (Table 1).

Overall, the percentage of persons with viral suppression within 3 months of diagnosis increased 6.4% (CI: 5.9-7.0) per year from 2017-2020 (Figure 1). By subpopulation, increases ranged from 3.7% (CI: 2.7–4.8) among White persons to 15.3% (CI: 1.4–31.1) among Native Hawaiian/other Pacific Islander persons per year. Viral suppression increased in areas of all population densities and in 22 of the 41 jurisdictions (Table 1).

**Conclusion:** Trends in prompt linkage to HIV medical care and early viral suppression showed progress overall; however, no changes since the baseline year of EHE were observed among some subpopulations and geographic areas. Accelerated efforts to increase access to HIV care and rapid start of treatment is urgently needed to meet national goals of reducing HIV transmission and eliminating disparities.

Linkage to HIV medical care within 1 month and viral suppression within 3 months of HIV diagnosis among persons aged ≥ 13 years during 2017–2020, by selected characteristics and area of residence – 41 jurisdictions

879 VIRAL SUPPRESSION AND COMMUNITY VIRAL LOAD AMONG MSM WITH HIV IN 23 US CITIES: 2017

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**Background:** ART adherence among MSM with HIV can lead to viral suppression (VS) in individuals and lower community viral load (CVL) in populations, reducing the potential for sexual transmission of HIV. Identifying
characteristics associated with VS and VL can inform programs aiming to improve VS and prevent HIV transmission among MSM.

**Methods:** In 2017, MSM in 23 U.S. cities—National HIV Behavioral Surveillance, 2017, N=2,149—were recruited via venue-based sampling to participate in National HIV Behavioral Surveillance. Participants included in our analysis were assigned male sex at birth, identified as male, were aged ≥ 18 years, ever had oral or anal sex with another man, completed the survey and rapid HIV testing, had an HIV-positive test result, and provided dried blood spots (DBS). HIV RNA viral load (VL) was obtained from DBS. We defined VS as having at least 1 result < 1000 copies/mL. We then used multivariate log-linked Poisson regression models to obtain adjusted prevalence ratios (aPR) and 95% confidence intervals (CI). For VL, when VL was below the lower level of quantification (LLOQ), we assigned a VL of one half of the LLOQ (832/2=416 copies/mL). We assessed CVL means, mean differences, and 95% CIs for key associations using a linear regression model on log_{10}-transformed VL counts. Final models were clustered for venue recruitment event and adjusted for city and key variables that were identified through a manual backwards elimination approach.

**Results:** VS was observed among 71.2% (1,530/2,149) participants. MSM who were Hispanic/Latino, had public health insurance, had private health insurance, and had an HIV care visit within the last 6 months were more likely to be virally suppressed (Table). VS was lower among those living below the federal poverty level. Estimated mean VL was 2.90 log_{10} copies/mL and was lower among Hispanic/Latinos, those with public or private health insurance, and those who had an HIV care visit in the last 6 months. CVL was higher among those living below the federal poverty level and those with an unmet need for healthcare due to cost in the last year.

**Conclusion:** Access to health insurance, routine HIV care visits, and not living below the federal poverty level were associated with VS and lower CVL. Programs to support health insurance access, retention in regular HIV care, and assistance with healthcare and daily living costs may be useful in assisting MSM with HIV in being virally suppressed and reduce the potential for population HIV transmission.


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<th>Characteristic</th>
<th>Mean(SD)</th>
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<td>Living below the federal poverty line</td>
<td>1.11 (SD=0.31)</td>
<td>Yes</td>
<td>1.11 (SD=0.31)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**880 HOSPITAL READMISSIONS AMONG ADULTS LIVING WITH HIV IN THE US**

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**Background:** Hospital readmission is a key indicator of quality-of-care. There are limited nationwide data characterizing readmissions among people living with HIV (PLHIV) in the era of universal HIV treatment. We described the contemporary risk of all-cause hospital readmission for adult PLHIV in the US.

**Methods:** Longitudinal data from the 2019 Nationwide Readmission Database, the largest public readmissions database which was developed by the Healthcare Cost and Utilization Project, were used to describe the risk of hospital readmissions among PLHIV. Adult PLHIV identified by ICD-10 codes who were discharged alive from their first recorded (index) hospitalization between January 2019 and November 2019 were included. We examined the probability of 30-day all-cause readmission since the discharge of index hospitalization. Age- and sex-adjusted risk ratios (aRR) of 30-day readmission were estimated by index admission characteristics (e.g., demographic, clinical and hospital factors) using modified Poisson regression models. Subgroup analyses were conducted stratified by sex. Survey weights were used to obtain nationally representative estimates.

**Results:** The weighted population included 55,812 HIV-related index hospitalizations, of which 68.9% were among males and the median age was 52 (IQR=39-59) years. The probability of 30-day readmission was 14.5% (n=13,848), and the top reason (except HIV) for rehospitalization was sepsis (10.3%). Older age and living in a zip code with a median household income < $48,000 were associated with a higher risk of readmission (Figure). Males and females had a similar risk [aRR=1.03 (95%CI=0.98-1.08)]. PLHIV with a higher clinical severity (APRDRG scores), admitted for non-elective reasons (vs. elective), and with comorbidities (e.g., dementia, substance use disorders, sepsicemia, hepatitis, and non-Hodgkin lymphoma) at index admission had a higher risk of readmission. In sex-stratified analyses, septicaemia index hospitalizations were associated with greater readmission risk among females [aRR=1.28 (95%CI=1.17-1.42)], but not among males [aRR=1.01 (0.95-1.08)]. Pregnant females at index hospitalization had a lower readmission risk than non-pregnant females [aRR=0.44 (0.35-0.53)].

**Conclusion:** Over 1 in 10 hospitalized PLHIV who were discharged alive were readmitted within 30 days. Readmissions disproportionally occurred among those with older age, from lower-income areas, and with comorbidities. Efforts are needed to mitigate potentially preventable readmissions among PLHIV.

881 HIV AND THE VETERANS CHOICE ACT: A GEOSPATIAL ANALYSIS IN GEORGIA, 2020-2022

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**Background:** One-quarter of all Veterans with HIV (VWH) — and three-quarters of rural VWH — live more than an hour’s drive from the nearest HIV clinic in the Veterans Administration (VA). Rural VWH who do not receive HIV care have suboptimal outcomes across the continuum of HIV care. In response, the 2014 Veterans Choice Act was enacted to expand healthcare access, which is purchased by the VA but provided by non-VA providers. Our objective was to examine the geospatial distributions of non-VA visits and detectable HIV viral load, and their associations with distance from the Atlanta VA Medical Center (AVAMC) among VWH.

**Methods:** Veterans diagnosed with HIV after August 1, 2014, who have had >1 visit at the AVAMC since September 15, 2020, and who reside in Georgia were included. Non-VA visits include any healthcare purchased by the VA and provided by non-VA providers. Our objective was to examine the geospatial distributions of non-VA visits and detectable HIV viral load, and their associations with distance from the Atlanta VA Medical Center (AVAMC) among VWH.

**Results:** The weighted population included 95,812 HIV-related index hospitalizations, of which 68.9% were among males and the median age was 52 (IQR=39-59) years. The probability of 30-day readmission was 14.5% (n=13,848), and the top reason (except HIV) for rehospitalization was sepsis (10.3%). Older age and living in a zip code with a median household income < $48,000 were associated with a higher risk of readmission (Figure). Males and females had a similar risk [aRR=1.03 (95%CI=0.98-1.08)]. PLHIV with a higher clinical severity (APRDRG scores), admitted for non-elective reasons (vs. elective), and with comorbidities (e.g., dementia, substance use disorders, sepsicemia, hepatitis, and non-Hodgkin lymphoma) at index admission had a higher risk of readmission. In sex-stratified analyses, septicaemia index hospitalizations were associated with greater readmission risk among females [aRR=1.28 (95%CI=1.17-1.42)], but not among males [aRR=1.01 (0.95-1.08)]. Pregnant females at index hospitalization had a lower readmission risk than non-pregnant females [aRR=0.44 (0.35-0.53)].
882 INTERNALIZED HIV STIGMA AND MENTAL HEALTH/SUBSTANCE USE OVER TIME

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Background: Internalized HIV stigma (IHS) may have significant impact on people with HIV (PWH). Our prior work suggests associations between IHS and increased HIV viral load are mediated by depression. Herein, we describe longitudinal changes in IHS and associations with mental health and substance use.

Methods: The CFAR Network of Integrated Clinical Systems (CNICS) is a longitudinal, multisite, cohort of PWH in care who complete tablet-based care assessments. At two different timepoints (T1 and T2) 1 year apart (from 2016-2020), IHS was assessed by 4 Likert scale response questions (low 1-5 high), with mean score and dichotomized score at ≤2 (no stigma) vs >2 used as primary exposures. We estimated adjusted rate ratios (ARR) of the association between IHS, depression, anxiety, and substance use at T1 and T2, adjusting for plausible confounders using conditional logistic regression (CLR) for dichotomized and Poisson regression (PR) for mean IHS score. We also fit autoregressive (AR) models with IHS parameterized as none, decreasing, constant, or increasing stigma (<2 to >2) from T1 to T2.

Results: 1,588 VWH met the inclusion criteria; of whom, 279 (18%) had a detectable HIV viral load. The number of non-VA visits exhibited significant spatial heterogeneity ($\chi^2=2030$, p = 0.002). VWH who reside outside of but surrounding Atlanta appear to have the highest average number of non-VA visits per year. However, for every 25-mile increase, it is estimated that the average number of non-VA visits decreases by 1.7 (p<0.001). The SPR of detectable HIV viral load varied considerably but was statistically insignificant ($\chi^2=91$, p=0.312). The observed prevalence of detectable HIV viral load is between two- and six-fold greater than what is expected in many parts of rural Georgia (e.g., SPR=4.3 in Jackson County, p=0.034). However, for every 25-mile increase, it is estimated that the number of detectable HIV viral load decreases by 1.5 (p<0.001).

Conclusion: VWH who reside in counties adjacent to the Atlanta metro area are more likely to receive non-VA healthcare, likely due to the >40-mile eligibility requirement and availability of such care. Non-VA healthcare may be less accessible in rural Georgia due to fewer resources and unaccounted socioeconomic factors. Telemedicine may be able to bridge this gap.

Figure 1. Geospatial distribution of non-VA purchased care visits and detectable HIV viral loads among Veterans with HIV who reside in Georgia and receive care at the Atlanta VA Medical Center, 2020-2022. Data are limited to Veterans diagnosed with HIV after the enactment of the Veterans Choice Act in August, 2020-2022.

883 RANDOMIZED TRIAL OF BRIEF ALCOHOL INTERVENTION FOR VIRAL SUPPRESSION AND ALCOHOL USE

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SEARCH Study Team 1University of California San Francisco, San Francisco, CA, USA, 2Infectious Diseases Research Collaboration, Kampala, Uganda, 3Kenya Medical Research Institute, Kisumu, Kenya, 4University of California Berkeley, Berkeley, CA, USA, 5National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 6Syracuse University, Syracuse, NY, USA, 7Makerere University, Kampala, Uganda

Background: Unhealthy alcohol use is a major contributor to viral non-suppression among persons with HIV (PWH), and a significant source of morbidity globally. It is unknown whether brief behavioral interventions to reduce alcohol use can improve viral suppression among PWH with unhealthy alcohol use in sub-Saharan Africa (SSA).

Methods: As part of the SEARCH study (NCT04810650), we conducted an individually randomized trial in Kenya and Uganda of a brief, culturally-adapted skill-based alcohol intervention among PWH with HIV viral non-suppression (viral load [VL] ≥400 copies/mL), missed visits, or out of care, and with self-reported unhealthy alcohol use (Alcohol Use Disorders Identification Test – Consumption [AUDIT-C] in the prior 3 months: ≥3/female; ≥4/male). The intervention included in-person counseling sessions at baseline and 3-months, and booster phone calls every 3-weeks in the interim. The primary outcome was viral suppression (VL < 400 copies/mL) at 24-weeks. Alcohol use at 24-weeks, assessed via self-report (AUDIT-C, prior 3 months) and phosphatidylethanol (PeM), an alcohol biomarker, was a secondary outcome. Results: 400 persons (197 intervention, 203 in control, 57% in Uganda) were enrolled from April 2021 through September 2021, with 94% analyzed.
at 24-weeks (6% moved from the study area). At baseline, 60% were virally suppressed. There was no difference in viral suppression between arms at 24-weeks: suppression was 83% in intervention and 82% in control arms (RR: 1.0, 95% CI: 0.93-1.0, p=0.40). Among PWH with baseline viral non-suppression (n=150), suppression was 73% in intervention and 64% in control arms (RR: 1.15, 95% CI: 0.93-1.43). Unhealthy alcohol use (AUDIT-C c≥3/female, ≥4/male or PEth≥50 mg/mL) declined from 100% at enrollment to 13% in intervention and 84% in control arms at 24-weeks (RR: 0.86, 95% CI: 0.79-0.94; p<0.001).

Effects on unhealthy alcohol use were stronger among women (RR: 0.70, 95% CI: 0.56-0.88; p=0.001) than men (RR: 0.93, 95% CI: 0.85-1.01; p=0.05). There were also intervention effects on high/high-risk alcohol use (AUDIT-C≥6 or PEth >200 mg/mL; RR: 0.84, 95% CI: 0.74-0.94; p=0.001).

Conclusion: In a randomized trial of 400 PWH with unhealthy alcohol use, a brief alcohol intervention reduced alcohol consumption but did not affect short-term viral suppression at 24-weeks. Brief alcohol interventions have the potential to improve the health of PWH in SSA by reducing alcohol use, a significant driver of HIV and associated co-morbidities.

884 12-MONTH CONSEQUENCES IN PEOPLE WITH HIV/SARS-CoV-2 COINFECTION: NATIONAL EHR COHORT
Yuanzhen Yue, Chen Liang, Sharon Weissman, Tianchu Lyu, Bankole Olatosoi, Xiaoming Li
National COVID Cohort Collaborative Consortium
University of South Carolina at Columbia, Columbia, SC, USA

Background: Long-term consequences of COVID-19 are well characterized in general populations. Yet it remains unclear how existing HIV infection attributes to the risks of long-term consequences in people with coinfecion of HIV/SARS-CoV-2. This study aims to examine the long-term consequences of people living with HIV (PLWH) at 12 months after the first SARS-CoV-2 infection.

Methods: Using the National COVID Cohort Collaborative (NCC), Electronic Health Records (EHR) sampled from 50 states and over 75 healthcare systems in the US, we constructed a cohort of PLWH with COVID-19 between March 1, 2020 and January 15, 2021, a historical control group (HIV individuals without COVID-19 between March 1, 2018 and January 15, 2019, two years predating the pandemic), and a contemporary control group (PLWH without COVID-19 between March 1, 2020 and January 15, 2021) to mitigate time/selection biases. The time of HIV infection was before March 1, 2020 for the cases and contemporary controls and, before March 1, 2018 for historical controls. The date of the first COVID-19 infection marked the start of a 12-month follow-up in the COVID-19 group. The start of follow-up in the contemporary controls was assigned by matching the same distribution of start dates of COVID-19 cases. We used logistic regression to examine odds ratios of health consequences at 12 months post COVID-19 comparing against contemporary and historical controls, respectively.

Results: We identified 5,619, 41,791, and 24,240 patients for COVID-19 cases, contemporary controls, and historical controls, respectively. The COVID-19 group had significantly higher odds in acute respiratory distress syndrome (OR: 3.45, 95% CI: 2.98, 3.99), hypertension (OR: 1.41, 95% CI: 1.29, 1.54), congestive heart failure (OR: 1.36, 95% CI: 1.14, 1.63), myocardial infarction (OR: 1.51, 95% CI: 1.22, 1.86), and diabetes (OR: 1.62, 95% CI: 1.42, 1.84), compared to contemporary controls. Odds in these outcomes were significantly higher when compared to historical controls (Figure 1).

Conclusion: This sentinel study for the first time reported elevated risks of multi-system dysfunction (i.e., respiratory, cardiovascular, and metabolic) among PLWH at 12 months post COVID-19. To our knowledge, it is the largest EHR cohort study assessing long-term consequences in PLWH. Our findings call for immediate attention to the post-COVID care among PLWH, including follow-up guidelines, care planning, and health policy tailored for PLWH.
A MULTICENTER STUDY OF COVID-19 INFECTION IN PEDIATRIC INTENSIVE CARE UNITS IN THE US

German A. Contreras, Gabriela Delbíanco, Michael Chang, Gilhen Rodriguez, Elizabeth Aguillera, James Murphy, Gloria Heresi
University of Texas at Houston, Houston, TX, USA

Background: To describe characteristics of COVID-19 infection among patients requiring admission to pediatric intensive care units (PICU) in the USA.

Methods: Observational study of COVID-19 infected patients admitted to PICUs in 27 US states between April 1, 2020 – May 1, 2021.

Results: Four hundred fifty-three patients were included; the majority were male (57%) and Hispanic (36%), with a median age of 10 years (IQR 4-15). 76% had 1 or more comorbidity.

Conclusion: Patient's or caregiver's reported sources for COVID-19 infection were household (88.8%) and anakinra (61%) were commonly used among individuals with MISC.

REINFECTIONS

Annalisa Saracino, Francesca D’Eugenio, Francesco Vladimiro Segala, Luca Carruba, Annalisa Saracino, Salvatore Bifulco, Francesco Muñoz-López, Antoni E. Bordoy, Ignacio Blanco, Elisa Matro, Pere-Joan Cardona, Roger Paredes, Bonaventura Clotet, Marc Noguera Julian, José Ramón Santos, Marta Massanella, Lourdes Mateu, Cristina Casañ, Ana Blanco-Suárez

Translational Research in Immunology and Ageing (TRIA)

Background: SARS-Cov-2 reinfections are more prevalent among women. Importantly, people with an undermined health status, independently of age, are more sensitive to reinfections, but in most of the cases no hospitalization was required. Finally, vaccination seems to have a short protective effect on reinfection.

ANEMIA AS A PREDICTOR OF POOR CLINICAL OUTCOME IN PATIENTS ADMITTED FOR COVID-19

Francesco Di Gennaro, Francesco Vladimir Segala, Luca Carruba, Anna La Carrubba, Diletta Agnello, Davide Fiore Bavarò, Mario Barbagallo, Nicola Veronese, Annalisa Saracino

University of Bari, Bari, Italy, University of Palermo, Palermo, Italy

Background: In respiratory infections, anemia is both a consequence of acute inflammatory syndrome and a predictor of mortality. In SARS-CoV-2 infections, the severity of the second infection may be caused by a diminished acquired immunity after the first infection.

Conclusion: SARS-Cov-2 reinfections are more prevalent among women. Importantly, people with an undermined health status, independently of age, are more sensitive to reinfections, but in most of the cases no hospitalization was required. Finally, vaccination seems to have a short protective effect on reinfection.

IDENTIFICATION OF CLINICAL FEATURES ASSOCIATED WITH SARS-Cov-2 REINFECTIONS

Francisco Muñoz-López, Antoni E. Bordoy, Ignacio Blanco, Elisa Matro, Pere-Joan Cardona, Roger Paredes, Bonaventura Clotet, Marc Noguera Julian, José Ramón Santos, Marta Massanella, Lourdes Mateu, Cristina Casañ, Ana Blanco-Suárez

Translational Research in Immunology and Ageing (TRIA)

Background: Over 600 million of COVID-19 cases have been reported. A remarkable fragment of these cases are reinfections, which are mostly explained by the genomic variability of the SARS-CoV-2 variants. However, little is known about other factors fostering these reinfections.

Methods: We recorded clinical and demographic data from subjects (N=3303, March 2020 – March 2022) with at least 2 PCR+ events separated by ≥90 days, analyzed by the Microbiology Department, Northern Metropolitan Clinical Laboratory from Germans Trias i Pujol Hospital (Spain). Data collected included: age, sex, comorbidities, adjusted morbidity group (GMA), hospitalization, symptomatology, NAAT (PCR, TMA) tests, antigen tests, serology, and vaccination. Temporal data was encoded using Python, and demographic characterization was performed under R.

Results: We identified 2344 cases of confirmed reinfections, where the 2 PCR+ events were separated by ≥90 days and a negative test was obtained between episodes. 72.2% of reinfection subjects were females with a median age of 45 IQR [28-63] years. Age density analysis showed three peaks at 24, 45, and 85 years, probably mostly composed of young people, who usually are less cautious, healthcare workers, and people living in nursing homes, respectively, being all of them groups prone to be tested. Regarding health status, 86.2% of participants had at least one chronic condition, with 40.5% of patients having chronic conditions in ≥4 systems based on GMA assessment. Interestingly, 75.2% of reinfectected subjects < 26 years had at least one chronic condition. 121 (4.2%) participants were hospitalized during a COVID-19 episode, highlighting 8.3% (N=10) of them hospitalized during the reinfection (half of them vaccinated before hospitalization), and 5% (N=6) of them during both infections. The severity of the second infection may be caused by a diminished acquired immunity after the first infection. Time between reinfections density analysis provided three peaks at ~200, ~400, and ~600 days, corresponding to the first and second peaks of the reinfection density curve in most cases. The severity of the second infection may be caused by a diminished acquired immunity after the first infection.

Conclusion: SARS-Cov-2 reinfections are more prevalent among women. Importantly, people with an undermined health status, independently of age, are more sensitive to reinfections, but in most of the cases no hospitalization was required. Finally, vaccination seems to have a short protective effect on reinfection.
ones. The association between anemia and the mortality was made using a Cox's regression analysis, adjusted, in two models, for the potential confounders and using a propensity score.

**Results:** Among the 1562 patients included in the analysis, prevalence of anemia was 45.1% (95% CI: 43.48%), and as shown in Table 1, were significantly older (p = 0.0001), reported more co-morbidities, and presented higher baseline levels of procalcitonin, CRP, ferritin and IL-6. As shown in Figure 1, patients with anemia reported a higher crude higher incidence of mortality compared to patients without this condition (Figure 1). Overall, the crude incidence of mortality was about four times higher in patients with anemia compared to those without. After adjusting for 17 potential confounders, the presence of anemia significantly increased the risk of death (HR = 2.68; 95% CI: 1.59-4.52) and of risk of severe COVID-19 (OR = 2.31; 95% CI: 1.65-3.24) (Table 2). The propensity score analysis substantially confirmed these analyses.

**Conclusion:** Our study provides evidence that, in patients hospitalized for COVID-19, anemia is both associated with a more pronounced baseline pro-inflammatory profile and higher incidence of in-hospital mortality and severe disease.

Figure 1. Survival curve by presence of anemia

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**Table 1 Outcomes by Dominant Variant**

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<th>Wave 1</th>
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<th>Wave 3</th>
<th>Wave 4</th>
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<th>Overall</th>
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<tbody>
<tr>
<td>ICU (%)</td>
<td>15.2%</td>
<td>17.7%</td>
<td>25.6%</td>
<td>14.9%</td>
<td>20.1%</td>
</tr>
<tr>
<td>KI (%)</td>
<td>7.7%</td>
<td>12.7%</td>
<td>12.9%</td>
<td>11.2%</td>
<td>11.6%</td>
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<tr>
<td>Mortality (%)</td>
<td>2.7%</td>
<td>2%</td>
<td>3.15%</td>
<td>2.9%</td>
<td>2.6%</td>
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**889 CLINICAL OUTCOMES BY SARS-CoV-2 VARIANT AT A SINGLE ACADEMIC MEDICAL CENTER**

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**Background:** SARS-CoV-2 continues to change over time due to genetic mutations and viral recombination.1 Given the changing landscape of COVID-19 variants and availability of COVID-19 vaccinations, disease severity during acute infection has also been variable. However, most research related to COVID-19 to date has not focused on evaluating differences in outcomes by the dominant variant and the impact it might have on post-acute sequelae of COVID-19 (PASC).

**Methods:** We developed a data mart of electronic health record data pertaining to COVID-19 in a single North American metropolitan health system (RUSH University Medical Center). Patients were selected for analysis if they had at least one documented infection of COVID-19. Date ranges were established per dominant variant, and the date of diagnosis was matched to variant. Variants were determined by the most prominent variant of concern (VOC) circulating in the city of Chicago. Variants were categorized by the following by date ranges: Wildtype +D614G (3/7/20-3/20/21), Alpha (3/21/21-6/19/21), Delta (6/20/21-12/11/21), Omicron BA.1 (12/12/21-3/19/22), Omicron BA.2 (3/20/22-6/18/22), and Omicron BA.4/BA.5 (6/19/22-present) (9/30/22). Subsequent clinical outcomes were examined, including hospitalization, intensive care unit admission, or death. We characterized our sample by conducting descriptive statistics including frequency and percent of outcome by variant.

**Results:** 44,499 patients were included in this analysis with 30.23% requiring hospitalization, 4.25% being admitted to intensive care unit (ICU), and 2.35% resulting in death. The greatest percentage of hospitalizations occurred with the Alpha variant at 41.88% (N=928), and the greatest percentage of ICU admissions (6.43%) and death (3.15%) occurred with the Delta variant. The latest Omicron variant (Wave 6) showed an increase in hospitalizations (35.18%), as compared to early Omicron waves (Wave 4 and 5) but maintained similar ICU rates. Death rates continued to decline during the Omicron waves (Table 1).

**Conclusion:** Although Alpha and Delta variants seem to have more severe outcomes compared to other variants, it is important to note that COVID-19 prevention, treatment access, and management continues to change, potentially influencing how outcomes may differ over time. Future work should determine factors to adjust for when examining variant-level differences.

**Table 1 Outcomes by Dominant Variant**

**890 EXCESS OF MORTALITY IN OLDER PEOPLE DURING THE COVID-19 PANDEMIC IN MOZAMBIQUE**

Inacio Mandomando1, Richard Sheppard2, Arsenia Masinga1, Rita Ernesto1, Auria de Jesus1, Augusto Messa Jr1, Ariel Nhacolo1, Arsenio Nhacolo1, Charufdin Sacoor1, Patrick Walker2, Alfredo Mayor4

1Centro de Investigação em Saúde de Manhica, Maputo, Mozambique, 2Imperial College London, London, United Kingdom, 3Centro de Investigação em Saúde de Manhica, Maputo, Mozambique, 4Universitat de Barcelona, Barcelona, Spain

**Background:** The impact of COVID-19 pandemic was apparently less severe in African continents, probably underestimated due to the limited testing capacities and access to health facilities, particularly in rural areas. Hospital and community surveillance of COVID-19 was established in Manhica District, rural Mozambique to understand the epidemic curve and natural history of SARS-CoV-2 including age-specific incidence of severe COVID-19 and reproduction number and effects of interventions through mathematical modelling.

**Methods:** Suspected cases visiting the Manhica District Hospital were screened for SARS-CoV-2 by RT-PCR. Four age-stratified (0-19, 20-39, 40-59 and ≥60 years, n=300 each) community-based serosurveys were conducted (Apr 2021-Feb 2022) to estimate the prevalence of antibodies (Abs) against SARS-CoV-2. We fitted a statistical model within a Bayesian framework, to estimate the expected levels of mortality in the absence of COVID-19 in adults aged 40+ using data from our reference category (15-39 year olds).

**Results:** Between Dec 2020 and Aug 2022, 31.2% of 1332 swabs tested positive for SARS-CoV-2, with high proportion among people aged 50-59 years (62.1%, 36/58). Abs against SARS-CoV-2 were detected in 28% (180/666) of subjects enrolled in survey one, which increased two and tri-fold, in surveys 2 and 3, respectively, to 64% (595/936) and 91% (700/768); remaining stable (91.3%, 1023/1121) in survey 3, remained positive 3 months later. Shifting age-patterns throughout the pandemic are consistent with a high impact of the disease particularly in older ages. Depending on assumptions made in our modelling, we estimate a cumulative excess mortality rates in adults aged 80+ and ≥60 years of between 8 and 17%.
of the pandemic that is largely not reflected in patterns of confirmed COVID-19 deaths. Quantitative estimates of shift in age-patterns throughout the pandemic. (A) Shows the fit of the model to age-patterns of mortality in the pre-pandemic period 2018-2020. This model is then used to generate the expected numbers of deaths in individuals aged 40+ throughout the pandemic (2020-2022). (B) excess deaths in the pandemic relative to the model, shown in (A), black lines and grey shaded regions show estimates assuming that declines in reported mortality in under 40s are due to declines in mortality (assumption 1), coloured show equivalent estimates assuming that declines in mortality in under 40s are due to declines in ascertainment (assumption 2). (C) Shows estimates from (A) as mortality per 1000 individuals within the age strata, (D) shows each excess mortality estimate as a proportion of the population within the age strata, with serosurveillance estimated from the first two cross-sectional surveys highlighted for reference.

891 HIV VIRAL LOAD SUPPRESSION AND RACIAL DISPARITIES DURING THE COVID-19 PANDEMIC IN NYC
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Background: Antiretroviral therapy is highly effective in achieving HIV viral load suppression (VLS) but requires sustained engagement in care. The COVID-19 pandemic disrupted medical care, and its impact on engagement in HIV care and VLS remains unclear. Health information exchanges (HIEs) enable examination of patient care across multiple health systems. We sought to leverage HIE data to examine the effect of pandemic-related disruptions in HIV care on VLS and to explore racial/ethnic disparities in VLS.

Methods: We performed a retrospective observational study of people living with HIV (PLWH) using de-identified data from Healthix, an HIE encompassing >20 million patients and 8,000 healthcare facilities in the greater New York City (NYC) region, between 1/1/2018 and 7/14/2022. We identified PLWH based on HIV viral load (VL) tests and HIV diagnosis codes (ICD and SNOMED).

We established two cohorts: PLWH engaged in care in 2020 with ≥1 VL test in 2019, 2020, and 2021 (Group A) and PLWH not engaged in care in 2020 with ≥1 VL test in 2019 and 2021 but 0 VL tests in 2020 (Group B). HIV VL outcomes were categorized as suppressed (< 200 copies/mL) or not suppressed (> 200 copies/mL) using the last VL in 2019, first VL in 2021, and last recorded VL.

Results: We identified 711,358 VL tests representing 81,122 patients at 249 facilities. Of these patients, 36,199 met our definition of PLWH. Of those, 12,448 were categorized as suppressed (< 200 copies/mL) or not suppressed (> 200 copies/mL) using the last VL in 2019, first VL in 2021, and last recorded VL. We compared proportions using X²-tests and fit a group-stratified logistic regression to examine the effect of race/ethnicity on VLS.

Conclusion: We identified 711,358 VL tests representing 81,122 patients at 249 facilities. Of these patients, 36,199 met our definition of PLWH. Of those, 12,448 were categorized as suppressed (< 200 copies/mL) or not suppressed (> 200 copies/mL) using the last VL in 2019, first VL in 2021, and last recorded VL. We compared proportions using X²-tests and fit a group-stratified logistic regression to examine the effect of race/ethnicity on VLS.

892 HIV AND CHRONIC COMORBIDITIES: MEDICATION ADHERENCE AND CLINICAL ENDPOINT COVARIATION
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Background: Evidence suggests negative monthly medication adherence trends during the COVID-19 era for patients with HIV (PWH) and multiple chronic conditions. However, it is unknown whether observed trends are associated with changes in outcomes of HIV care before and during the COVID-19 era.

Methods: Adult PWH with type 2 diabetes, hypertension, and/or hypercholesterolemia were identified in a US mid-Atlantic integrated health system. Multivariable population-averaged panel general estimating equations were used to assess the relationship between medication adherence (i.e., accepted dichotomous thresholds for optimal proportion of days covered (PDC)) for four medication groups: antiretrovirals (ART), diabetes medications (DMs), renin-angiotensin antagonists (RASMs), and statins (SMs) and their related clinical endpoints (i.e., viral load (VL); copies/mL, HbA1c, systolic and diastolic blood pressure (SBP, DBP; mmHg), and total cholesterol (TC; mg/dl)) during a 37-month longitudinal observation period (9/2018–9/2021). Covariates included demographics, number of medication groups, COVID-19 era (starting 3/1/2020), and a COVID-19/PDC interaction term.

Results: The cohort (n=543) was predominantly 51-64y (59.30%), Black (73.11%), male (69.24%), and privately insured (65.38%). All patients were prescribed ART with 75.32% co-prescribed SmDs; followed by RASMs (42.73%); and DMs (32.60%). ART PDC≥0.9 was associated with decreased odds of VL≥200 copies/mL [aOR=0.77, 95% CI: 0.63-0.94]. For DMs, RASMs and SMs, PDC≥0.8 was not associated with the clinical endpoints of HbA1c≥7.0% [aOR=0.99, 95% CI: 0.94-1.04], SBP≥130 mmHg [aOR=1.03; 95% CI: 0.93-1.14], DBP≥80 mmHg [aOR=1.05, 95% CI: 0.94-1.16] or TC≥200 mg/dl [aOR=1.00, 95% CI: 0.96-1.04], respectively. The COVID-19 era (3/2020 to 9/2021) was associated with increased odds of SBP≥130 [aOR=1.22, 95% CI: 1.01-1.48], but not for DBP≥80 mmHg [aOR=1.05, 95% CI: 0.85-1.28], VL≥200 copies/mL [aOR=1.01, 95% CI: 0.67-1.52], HbA1c≥7.0% [aOR=0.99, 95% CI: 0.88-1.11], and TC≥200 mg/dl [aOR=0.95, 95% CI: 0.86-1.04]. No interactions between COVID-19 era and PDC on clinical endpoints were observed.

Conclusion: ART adherence was associated with viral suppression in PWH, but there were no observed associations between DM, RASMs, and SM adherence and their respective clinical endpoints. With the exception of a direct relationship between the COVID-19 start date and SBP, the COVID-19 era was not associated with variations in VL, HbA1c, DBP, and TC clinical endpoints.

893 HIGH PANDEMIC-RELATED MORTALITY AMONGST PEOPLE WITH HIV IN SASKATCHEWAN, CANADA
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Background: Saskatchewan, a Canadian Prairie province, faces a complicated HIV epidemic characterized by high rates of transmission due to injection drug use (IDU) and disproportionate representation of younger persons, women, and persons of Indigenous ethnicity. HIV incidence in Saskatchewan in 2021 was 19.7 per 100,000, 4.5 times higher than the Canadian average. Concurrently, during the COVID-19 pandemic, the recreational use of synthetic opioids such as fentanyl increased, leading to high numbers of overdose events & deaths.
We characterized the difference in cascade of care outcomes & mortality amongst people with HIV (PWH) living in southern Saskatchewan during the COVID-19 pandemic.

**Methods:** We conducted a retrospective cohort study for all PWH cared for in the Infectious Diseases Clinic (IDC) at Regina General Hospital between December 31/19 and June 10/22. Age, sex, ethnicity & primary mode of HIV acquisition were collected from the IDC database, along with cascade of care & mortality data. Deaths, including most likely cause of death were characterized via individualized case review.

**Results:** On December 31/19, IDC cared for 518 PWH. This increased to 585 by June 10/22. Amongst the current cohort, 245 (42%) were female, 163 (28%) were ≤ 35 years old, 306 (52%) were Indigenous, and 318 (54%) had acquired HIV through IDU.

Cascade of care indicators worsened during the COVID-19 pandemic. 58.1% of the cohort were retained in care & 76.1% virally suppressed (HIV RNA ≤ 200 copies/mL) in December 2019, decreasing to 51.3% retained (p=0.02) & 68.8% suppressed (p=0.06) by June 2022.

There were 80 deaths during the study period, representing 15.4% of the cohort from the end of 2019. Most deaths (49, 61.3%) were due to suspected or confirmed drug overdose. 10 (12.5%) additional deaths occurred due to complications from IDU (i.e., sepsis). No deaths were directly attributable to COVID-19. Most who died acquired HIV from IDU (69/80, 86%).

**Conclusion:** We describe intersecting epidemics of HIV and IDU disproportionately affecting high-risk populations, leading to significant morbidity & mortality during the COVID-19 pandemic. Contributing factors may have included disruption of safe opioid supply and reduced access to harm reduction services due to COVID-19. Comprehensive prevention-level harm reduction and addictions management strategies are urgently needed to reduce morbidity & mortality from drug use among PWH in Saskatchewan.

**894 IMPACT OF THE COVID-19 PANDEMIC ON MALARIA SERVICES IN UGANDA: A TIME SERIES ANALYSIS**

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1University of Bari, Bari, Italy, 2Doctors with Africa CUAMM, Kampala, Uganda, 3Istituto Superiore di Sanità, Rome, Italy, 4St. John’s XXIII Hospital Aber, Aber, Uganda, 5Doctors with Africa CUAMM, Kampala, Uganda, 6Doctors with Africa CUAMM Kampala, Uganda, 7Doctors with Africa CUAMM, Padua, Italy, 8Doctors with Africa CUAMM Kampala, Kampala, Uganda.

**Background:** Pregnancy is both a risk factor for P. falciparum infection and development of severe malaria and, in Uganda, its control relies heavily in the administration of intermittent preventive treatment with sulfadoxine-pyrimethamine (SP-IPTp) during antenatal care visits (ANC). COVID-19 pandemic severely impacted health systems globally. This study aims to assess trends in delivering malaria in pregnancy related healthcare services before and during Covid-19 in thirty health facilities in Northern Uganda.

**Methods:** Interrupted time series study comparing two periods: I) pre-Covid-19 (January 2018 to February 2020) and II) Covid-19 (from March 2020 to December 2021) period. Data were sourced from the District Health Information Management System II (DHIMS2) routinely collected indicators. Comparisons between the two periods were computed with a jointpoint regression model and Annual Average Percentage Changes (AAPC) were calculated.

**Results:** The study involved data collected by 30 health facilities, 30 health facilities in Northern Uganda – including one hospital - with a catchment area of 506,276 inhabitants and an estimated number of pregnancies ranging from 21,440 to 23,315. Cotivid cumulative cases and deaths for Oyam districts are reported in Figure 1. As shown in Figure 2, during COVID period we found a significant reduction in the number of women accessing to at least 4 antenatal care (ANC) visits and taking at least three doses of intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine. The total number of pregnant women receiving Artemether-Lumefantrine for nonsevere malaria or being hospitalized for severe malaria, along with the total number of institutional deliveries and stillbirths followed kept following the trend recorded prior to the pandemic.

**Conclusion:** The present study shows that, despite the international call for prioritization of maternal and reproductive health service delivery during COVID-19 pandemic, in Uganda, the essential care for malaria in pregnancy have been disrupted. This is concerning, as the failure to increase the delivery of SP-IPTp may impact malaria-related mortality.

Jointpoint regression model for selected indicator

**895 NEW HIV DIAGNOSES IDENTIFIED FROM LINKAGE TO CARE AFTER MPX INFECTION, TEXAS 2022**

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**Background:** HIV surveillance collects data from laboratory reports submitted to the health department. Staff evaluate lab results to determine follow-up activities. Following the 2014 HIV Surveillance Case Definition, all persons whose lab results are indicative of a new HIV diagnosis are contacted for partner services while surveillance staff collect information from the diagnosing provider. Individual lab results are insufficient to make a case determination. Disease intervention specialists are dispatched to offer follow-up testing, but patients can decline. Patients are occasionally lost to follow-up with only a positive screening test reported. The first mpox case in Texas was identified on June 7, 2022, and the U.S. Department of Health and Human Services declared mpox a public health emergency on August 4. Matching cases between the HIV/STD and mpox registries began in September. Matching mpox cases with the HIV registry enabled staff to identify persons with incomplete HIV case reporting and prioritize follow-up.

**Methods:** Matched was conducted with all persons reported to Emerging & Acute Infectious Disease Unit (EAIDU) with confirmed and probable mpox. Person identifiers reported through routine electronic reporting were used for the match, including first and last name, birth date, birth sex, and address. Mbox cases reported to EAIDU thru November 26 were matched to the HIV registry. The HIV dataset included all persons diagnosed with HIV and with preliminary positive screening tests. Data was matched using statistical software SAS 9.4.

**Results:** Of the 2,826 mpox cases reported to DSHS thru November 28, 2022, 1,413 (50%) matched to the people who had a documented case of HIV meeting the 2014 case definition. During the 4 months of matches, a total of 51 new HIV cases were identified through matching mpox and HIV data. These people had preliminary HIV testing but did not have complete case reporting for HIV. After mpox linkage to care, they received public health follow-up confirming their HIV status and a complete an HIV surveillance report. People identified as comorbid cases had applications for AIDS Drug Assistance Program and Care Services expedited allowing clients to receive services.

**Conclusion:** Utilizing disease data sources other than HIV/STD can assist in identifying individuals who did not receive an HIV diagnosis, were lost to follow-up, or did not link to HIV care after diagnosis allowing prioritization for HIV/STD public health follow-up and resource assistance.

**896 MPXV VIRUS IN THE PHARYNX OF MEN HAVING SEX WITH MEN: A CASE SERIES**

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1IRCCS Fondazione San Gerardo di Monza, Monza, Italy, 3Università Politecnica San Matteo Foundation, Pavia, Italy, 9Università degli Studi di Milano-Bicocca, Monza, Italy

**Background:** The recent outbreak of monkeypox virus (Mpx) in Europe and North America was predominantly sustained by sexual transmission among men who have sex with men (MSM). Although vital virus has been recovered from sexual fluids, different sources of transmission are possible. Therefore, the dynamic of the virus in other organs and fluids, such as in the pharynx, merits to be investigated.

**Methods:** We describe a case series of patients with Mpx recruited in an urban STI center in Lombardy (Italy) between May and Aug 2022. We collected
data on demographics, transmission, and clinical presentation using a standardized form. Skin lesion and oropharyngeal swabs were collected from all patients at baseline and tested for the presence of the Mpx using the RealStar Orthopoxvirus PCR Kit 1.0 (Altona diagnostics). Since July, all patients with a positive swab underwent weekly test of skin lesions and oropharyngeal swabs until both tests resulted to be negative. **Results:** 15 patients, all MSM, 40% HIV-positive, were included. All patients but one reported recent unprotected sexual activity. Oropharyngeal symptoms (pharyngodynia or odynophagia) were reported only by one third of the patients and lesions in the oral cavity were present only in 20%. These and other characteristics are showed in Table 1.

Mpx was identified in 14/15 patients from skin lesions while in one patient it was identified only in the oropharyngeal swab. Overall, oropharyngeal swabs tested positive in 13/15 (86.7%) of the patients. Lower cycle threshold (Ct), indicating higher viral load, was measured in skin than in pharynx swabs (mean Ct 18.1 [95%CI 14.8-21.5] vs. 24.2 [21-27.5]). Nonetheless, among 7 patients followed prospectively until clinical and virological cure, Mpx PCR positivity persisted, on average, 5.3 days longer in pharynx than on skin. In one immunosuppressed patient (due to previous bone marrow transplantation), oropharyngeal swabs remained positive for 80 days (55 days beyond skin lesion resolution). No cases of transmission in the household, apart from one transmission to the cohabiting sexual partner, occurred.

**Conclusion:** Our findings suggest that Mpx can be recovered from the pharynx in absence of symptoms or signs of local involvement and that it can persist in the pharynx for long. Routine testing of the oropharyngeal swab among suspect cases and exposed individuals may contribute to increase diagnostic yield. Whether replication in the oropharynx may contribute to disease transmission needs to be assessed.

Table 1: Patient characteristics

**Table 1:** Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%) 15 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 (17-34)</td>
</tr>
<tr>
<td>Male history of sex with Mpx</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td></td>
</tr>
<tr>
<td>HIV-positive</td>
<td></td>
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<tr>
<td>Immunosuppressed</td>
<td></td>
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<tr>
<td>Anorectal lesion</td>
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<tr>
<td>Proctitis</td>
<td></td>
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<tr>
<td>Number of lesions in absence</td>
<td></td>
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<tr>
<td>Number of lesions in presence</td>
<td></td>
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<tr>
<td>Number of immunocompromised lesions</td>
<td></td>
</tr>
<tr>
<td>Sexually transmitted</td>
<td></td>
</tr>
<tr>
<td>HIV-positive</td>
<td></td>
</tr>
<tr>
<td>Positive oropharynx</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td></td>
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<tr>
<td>Sexually transmitted</td>
<td></td>
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<tr>
<td>Number of lesions in absence</td>
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<tr>
<td>Number of lesions in presence</td>
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897 MPOX AMONG MSM IN THE NETHERLANDS PRIOR TO MAY 2022, A RETROSPECTIVE STUDY

Elske Hoornenborg,1 Henry de Vries,1 Hannelore Götz,1 Sylvia Bruisten,1 Annemiek van Der Eijk,2 Maria Prins,3 Bas Oude Munnik,3 Matthijs Welkers,3 Marcel Jonges,3 Richard Molenkamp,2 Benda Westerhuis,2 Daphne Mulders,4 Leonard Schule1, Maniken van Der Liubben,1 Marion Koopmans4

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**Background:** Since May 2022 over 20.000 monkeypox (mpx) cases have been reported from 29 EU/EEA countries, predominantly among men who have sex with men (MSM). With over 1200 cases and a crude notification rate of 70.7 per million population, the Netherlands was in the top 5 European countries most affected. The first national case was reported from May 10th; yet potential prior transmission remains unknown. We therefore performed a retrospective study to elucidate whether undetected transmission of human monkeypox virus (hMPXV) occurred prior to the first reported cases in the two largest cities in the Netherlands, Amsterdam and Rotterdam.

**Methods:** Data from the two largest Centers for Sexual Health in the Netherlands were used. We retrospectively tested stored samples for hMPXV using an in-house developed and validated qPCR. Stored samples comprised anorectal samples that were positive for Chlamydia or Gonorrhoea (Ct/Ng) and all collected ulcer samples. Whole genome sequencing was performed for hMPXV positive samples. For phylogenetic analysis we used all available Genbank sequences and added the Dutch strains generated as part of this study.

**Results:** We tested 262 (169 in Amsterdam; 93 in Rotterdam) anorectal samples that were positive for Ct/Ng and 137 ulcer samples (125 Amsterdam, 12 Rotterdam) collected between February 14 and May 18, 2022 on the presence of hMPXV. Two hMPXV positive samples were identified, one from an anorectal sample and one from an ulcer sample, both collected in the first week of May (week 18), 2022. Both samples were from MSM, one had symptoms of proctitis and one had multiple ulcers. The anorectal hMPXV positive sample was successfully sequenced. This sequence, like all other Dutch sequences belonged to the clade lb cluster (B1) with a close relation to international strains of hMPXV (figure).

**Conclusion:** The first mpx cases in the Netherlands coincided with the first cases reported in the United Kingdom, Spain and Portugal. We found no evidence of widespread hMPXV transmission in Dutch sexual networks of MSM prior to May 2022. Likely, the hMPXV outbreak expanded across Europe within a short period in the spring of 2022 in an internationally highly intertwined network of sexually active MSM.
**Conclusion:** SGM YAA in Illinois overwhelmingly reported reducing sexual contact due to the mpox outbreak. Vaccinated individuals were more likely to report reduced sexual activity and a greater number of prophylactic activities. Thus, sex-positive and harm reduction messaging strategies are likely to be more effective than abstinence-only prevention, which may further stigmatize marginalized groups.

Demographic Characteristics and Bivariate Associations with Sexual Contact (n = 317).

**SEXUAL BEHAVIOR REDUCTION DO NOT EXPLAIN DECREASED MPXV INCIDENCE AMONG PrEP USERS**

Roberto Rossetti,1 Daniele Calzavara,2 Massimo Cernuschi,3 Anna De Bona,4 Alessandro Tavelli,5 Simona Bossolasco,6 Camilla Muccini,3 Daniele Tesoro,4 Davide Moschese,7 Giovanni Mulè4, Antonella D’Arminio Monforte8

1ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, 2Milano Checkpoint, Milan, Italy, 3San Raffaele Scientific Institute, Milan, Italy, 4ASST Santi Paolo e Carlo, Milan, Italy, 5Icona Foundation, Milan, Italy, 6Ospedale San Raffaele, Milan, Italy, 7Luigi Sacco University Hospital, Milan, Italy, 8University of Milan, Milan, Italy

**Background:** The Italian monkeypox (MPX) outbreak involved essentially the Milan area starting from mid-June 2022. As observed in other European regions, new cases decreased significantly by October but no reasons for this epidemiologic trend have been established yet. We aimed to assess whether reduction in sexual activity and at-risk behaviors and/or vaccination might explain this finding in PrEP users attending a community-based service.

**Methods:** Milano Checkpoint provides assistance to the largest Italian cohort of PrEP users. At each visit clients are tested for sexually transmitted infections (STIs) and fill self-administered questionnaires about their behavior in the previous 3 months. Subjects with a visit in July to November 2022 were selected: overall and condomless sexual intercourses, chemsex practices, and STIs incidence were compared to what registered in the previous visit. Descriptive statistics and non-parametric tests (Pearson's Chi-square, Mann-Withney U, McNemar exact, and Wilcoxon signed-rank) were used to compare groups, while logistic regression models with potential covariates were built to describe factors associated to change in sexual behavior. Logistic regression analyses were used in subsequent bivariate and multivariate logistic regression analyses.

**Results:** We selected 435 individuals: Table 1 describes features of study population. Smallpox vaccine was available from the second half of August and only a minority (26.2%) completed the full course. A reduction in the number of sexual contacts was observed in 174 (40.0%) PrEP users, but the majority did not change the number of sex acts during the MPX outbreak: the overall number of sexual contacts arose from 11 (IQR 5-25) to 12 (IQR 5-26) in the epidemic months (p=0.070). Condomless intercourses and use of chemsex did not change (p=0.459 and p=0.766, respectively). The incidence of STIs was 87.3 per 100 PYFU in the pre-epidemic versus 84.8 per 100 PYFU in the epidemic period (IRR 1.03, 95% CI 0.80-1.32, p=0.813). The only factor associated to reduction in sexual activity was a lower level of education (OR 0.69, 95% CI 0.54-0.86, p=0.001). Sexual behavior did not change after vaccination (p=0.593) nor a diagnosis of MPX (p=0.856).

**Conclusion:** Both reduction of risky sexual behavior and MPX vaccination do not explain the vanishing of epidemics. Saturation of high-risk groups or hesitancy to contact health facilities to avoid quarantining policies should be investigated.

**STIGMA RELATED TO HUMAN MPXV VIRUS AMONG MSM IN THE US, AUGUST 2022**

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**Background:** Stigma related to identity, behavior, and HIV status can manifest in multiple domains of life. In the 2022 mpox outbreak, gay, bisexual, and other men who have sex with men (MSM) and non-Hispanic Black and Hispanic men have represented overly large proportions of reported cases. Because these groups also regularly face stigma in their lives, there was potential for messaging about the mpox outbreak to increase stigmatization for these already marginalized groups. However, CDC sought to promote non-stigmatizing, sex positive harm reduction messages from the beginning of the outbreak.

**Methods:** To understand prevalence and impact of mpox-related stigma amongst MSM in the United States, we conducted a study within the American Men's Internet Survey, which enrolled 824 cisgender MSM aged 15+ from August 5-15, 2022. We administered 10 mpox-related stigma items; we retained 9 after exploratory factor analysis and pooled these 9 into a binary variable for “any reported stigma” which was used in subsequent bivariate and multivariate logistic regression analyses.

**Results:** 1.9% (n=15) of participants felt excluded from their family because of fear they may have mpox, with 1.1% (n=9) unsure. 1.8% (n=14) of participants felt excluded from friends or were unsure 1.8% (n=14) of participants felt excluded from friends or were unsure 1.8% (n=14) of participants felt excluded from friends or were unsure. Regarding discriminatory remarks or gossip from family, 3.5% (n=28) felt some level of discrimination, with another 3.8% (n=30) unsure. Overall, 1.3% (n=10) felt worried about going to their doctor because of being diagnosed with mpox. In total, 1.0% (n=8) of participants reported being verbally harassed because someone thought they had mpox, with another 0.6% (n=5) unsure. Overall, 6.6% (n=54) reported experiencing any mpox-related stigma. In bivariate analyses, reporting any mpox-related stigma was associated with knowing someone who was vaccinated for mpox (OR=4.15; 95% CI=1.12, 8.69), having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69), and having an mpox diagnosis (OR=4.15; 95% CI=1.12, 8.69). Multivariable logistic regression models with potential intersecting determinants such as race/ethnicity, age group, and region did not significantly affect the presented relationships.

**Conclusion:** There was low overall prevalence of mpox-related stigma among MSM in August 2022. These data suggest that messages developed by CDC and others about mpox and how to protect oneself from mpox infection did not lead to widespread stigma for this sample of MSM in the US.
901 HIGH LEVEL OF MPOX KNOWLEDGE AND STIGMA AMONG LGBTQIA+ COMMUNITIES IN BRAZIL
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The IHI-Icruzer Mpxx Study Group
Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Background: Globally in 2022, Brazil ranks second in number of mpxx cases and deaths. Stigma and discrimination, against the LGBTQIA+ community, might result in structural barriers that impact access to health services, leading to undertesting and underreporting of cases. We aim to evaluate community knowledge of mpxx, and to describe sociodemographic and behavioral aspects according to self-reported mpxx diagnosis.

Methods: Cross-sectional online survey conducted among adults (>18 years) living in Brazil through advertisements on Scruff, Grindr, Instagram, and Facebook. Questions addressed sociodemographics; mpxx knowledge, symptoms, and diagnosis; sexual behavior; HIV testing; and STI diagnoses. For comparative statistical analysis, we used chi-square and Kruskal-Wallis tests.

Results: From October to November 2022, 6236 participants completed the survey: 5686 (91.2%) cis-man, 2524 (40.4%) non-binary, 2474 (40.4%) cis-women, 150.2% trans-man and 150.2% trans-women. Most were gay/bi/pansexual (6032; 96.7%), white (3877; 62.2%), had college education (4902; 78.7%), and low/middle income (4070; 73.0%). Most had heard of mpxx (6044; 96.9%) and reported willingness to take mpxx vaccine (5908; 95.1%). Overall, 324 (5.6%) reported a mpxx diagnosis; 318 of them (98.1%) reported lesions, 178 (56.0%) local pain and 316 (99.4%) attended health facilities for investigation. Among participants who reported no mpxx diagnosis (N=5912), 4974 (84.1%) reported knowledge of mpxx, 288 (4.9%) had a suspicious mpxx lesion, but only 54.9% of those (n=158/288) attended a health facility for investigation. Participants reporting mpxx diagnosis compared to those not diagnosed were younger (median 34 [IQR:30-39.2] vs. 37 [IQR:31-44]; p<0.001), reported sex partners with suspicious/confirmed mpxx (34.6% vs. 3.5%; p<0.001) and more sexual contacts (Table). HIV testing in the prior 3 months (74.1% vs. 42.1%; p<0.001), current PrEP use (47.8% vs. 24.4%; p<0.001) and any STI diagnoses (25.0% vs. 10.6%; p<0.001) were higher among those reporting mpxx diagnosis. Participants reporting mpxx diagnosis more frequently reported changes in sexual behavior after mpxx onset. HIV prevalence differed by report of mpxx diagnosis (37.7% vs. 22.9%; p<0.001). The majority affirm that LGBTQIA+ communities are being stigmatized due to mpxx (5258; 84.4%).

Conclusion: Our results show high rates of mpxx knowledge in the LGBTQIA+ communities. Expand our data to gender competent care is critical to avoid underdiagnosis and fight stigma and discrimination.

902 CHARACTERISTICS AND DISPARITIES AMONG HOSPITALIZED PERSONS WITH MPOX IN CALIFORNIA

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Background: In the 2022 outbreak of mpxx, reported hospitalization rates have ranged from 2%–13%. Severe clinical manifestations have been reported in persons coinfected with mpxx and HIV. However, data describing sociodemographic characteristics and comorbidities among hospitalized persons with mpxx are limited. We compared demographic characteristics, highlighting socioeconomic disparities and clinical characteristics, including HIV infection, among persons with mpxx who were and were not hospitalized in California.

Methods: We included mpxx cases reported in California from May 17 through September 8, 2022. All analyses were stratified by HIV status. Census tracts of residence of mpxx cases were matched to the California Healthy Places Index (HPI), which categorizes neighborhoods based on characteristics that influence health of residents, with lower scores relating to fewer opportunities for residents of these communities to lead healthy lives. Fisher’s exact tests and Wilcoxon rank sum tests were used to compare groups.

Results: Of 3241 persons with mpxx, 1317 (41%) had HIV and 119 (4%) were hospitalized. Among those with HIV 68(5%) were hospitalized and 51(3%) without HIV were hospitalized. Hospitalization was commonly for oropharyngeal (25(21%)) or rectal symptoms (19(16%)), and bacterial infections (19(16%)). Among those with HIV, more hospitalized than non-hospitalized persons lived in the lowest HPI quartile (26(42%) versus 279(23%), p<0.01); no difference was seen among those without HIV. In those with HIV, having CD4 < 200 cells/μL (9(14%) versus 46(4%)) or a non-suppressed (>200 copies/ml) viral load (27(42%) versus 121(9%)) was associated with hospitalization (p-values<0.01). For those with HIV who were hospitalized, 13(36%) with non-suppressed viral loads and 4(4%) with CD4 < 200 cells/μL lived in the lowest HPI quartile. Among those without HIV, having diabetes (36%) versus 312%, p<0.001) or exfoliative skin conditions (510% versus 593%), p<0.001 was associated with hospitalization.

Conclusion: Among persons with mpxx and HIV, more hospitalized cases had uncontrolled HIV and lived in communities with fewer opportunities to lead healthy lives. Among persons with mpxx and without HIV, more that were hospitalized had diabetes or exfoliative skin disorders. Vaccination and rapid access to testing and treatment should be prioritized in these groups.

903 CD4 COUNT < 350 CELLS/MM3 INCREASES RISK OF HOSPITALIZATION WITH MPOX IN PWH
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Background: Mpxx is typically a self-limited infection; however, HIV-associated immunosuppression increases the risk of severe illness. For persons with HIV (PWH), correlates of risk for severe illness, such as illness severe enough to warrant hospitalization, have not been well characterized. Such data could help determine which PWH with mpxx should be prioritized for close monitoring and care including early or emergic tecovirimat treatment.

Methods: We characterized the HIV status of all reported cases of mpxx in Georgia from 5/31/2022–10/31/2022 by linking surveillance data for mpxx cases with HIV cases, including HIV laboratory results. We used a retrospective cohort design and a modified Poisson regression model with a log-link and robust variance estimates to calculate relative risks (RRs) for hospitalization with mpxx. The predictor variable captured 1) most recent CD4 cell count (CD4) in the year prior to mpxx onset. The predictor variable captured 2) engagement in care defined as any HIV monitoring and care including early or empiric tecovirimat treatment.

Results: We characterized the HIV status of all reported cases of mpxx in Georgia from 5/31/2022–10/31/2022 by linking surveillance data for mpxx cases with HIV cases, including HIV laboratory results. We used a retrospective cohort design and a modified Poisson regression model with a log-link and robust variance estimates to calculate relative risks (RRs) for hospitalization with mpxx. The predictor variable captured 1) most recent CD4 cell count (CD4) in the year prior to mpxx onset. The predictor variable captured 2) engagement in care defined as any HIV monitoring and care including early or empiric tecovirimat treatment.

Results: Among the 1,921 mpxx cases in Georgia, 1,851 (96%) were among cisgender men, and 1,124 (59%) were among PWH of whom 214 (10%) had a CD4 count < 350 cells/mm3 and 189 (17%) had an unsuppressed viral load (>200 copies/ml) in the year prior to mpxx onset. Among 121 persons reported as hospitalized with mpxx to GDPh, 84 (69%) were PWH, of whom 32 (26%) had CD4 < 350 cells/mm3 and 15 (12%) had no CD4 or VL results in the year prior to PWH. Common reasons for hospitalization included pain control (37%), breathing problems (13%), and treatment of a secondary infection (11%). Among PWH, persons with low CD4 count had increased risk of hospitalization starting around CD4 < 350 cells/mm3 (Figure). Risk for hospitalization among PWH with
CD4 > 350 cells/mm$^3$ was similar to that for persons without HIV (RR 1.0, 95% confidence intervals [CI] 0.6-1.5); however, PWH with CD4 < 350 cells/mm$^3$ were more likely to be hospitalized (RR 3.2, 95% CI 2.1-5.1). PWH without recent HIV laboratory results were also more likely to be hospitalized (RR 2.4, 95% CI 1.3-4.2).

Conclusion: PWH were more likely to be hospitalized with mpox if their most recent CD4 was < 350 cells/mm$^3$ or if they were not engaged in care (using laboratory criteria in the past year as a proxy). For PWH diagnosed with mpox who have CD4 < 350 cells/mm$^3$ or who are not engaged in HIV care, clinicians should closely monitor illness and consider early treatment with medical countermeasures such as tecovirimat.

Crude risk of hospitalization with mpox among persons with HIV by CD4 count produced with locally estimated scatterplot smoothing, shaded area is 95% confidence interval

904 MPXV VIRUS INFECTION IS MORE SEVERE IN PATIENTS WITH UNCONTROLLED HIV INFECTION

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Methods: National case-series study. Patients from 18 Spanish hospitals, with PCR-confirmed MPXV infection since April 27th to September 30th were included in this study. The duration of the clinical course was computed from the onset of symptoms until mucocutaneous lesions complete clearance or MPXV infection-related complications resolution. Disseminated disease was defined as the presence of mucocutaneous lesions involving 6 or more areas of the body surface. Severe complications included extensive superinfection of skin lesions without response to treatment, pain refractory to non-opioid analgesia, sepsis, odynophagia with obstructive sensation, myopericarditis, gastrointestinal bleeding, encephalitis, or ophthalmologic complications. The main outcome was the development of severe MPXV disease, defined as: i) duration of the clinical course ≥ 21 days; or, ii) disseminated disease; or, iii) emergence of severe complications, or iv) requirement of hospital admission.

Results: 1,028 individuals were included. Overall, 928 (90%) were MSM. 448 (43%) were PLWH, of whom 26 (2%) had a CD4 cell count < 350 cells/mm$^3$. HIV viral load ≥ 1,000 cp/mL was found in 19 (4%) PLWH. 18 of them (94%) were not on ART. Severe MPXV disease was observed in 16 (62%) PLWH with CD4 < 350 cells/mm$^3$, 164 (41%) PLWH with CD4 ≥ 350 cells/mm$^3$ and 208 (40%); i.e., 61% PLWH and CD4 < 350 cells/mm$^3$ vs. 372 (40%); p < 0.032, of the remaining study participants showed severe disease. Regarding plasma HIV viremia, 14 (74%) PLWH with HIV-RNA ≥ 1,000 cp/mL showed severe disease vs. 174 (41%) PLWH with plasma HIV-RNA load < 1,000 copies/mL. 222 (38%) individuals without HIV infection (p=0.008). In multivariate analysis, adjusted by age, sex, CD4 cell count and HIV viral load at the time of MPXV infection, only plasma HIV-RNA ≥ 1,000 cp/mL was associated with a greater risk of developing severe MPXV disease among PLWH (adjusted OR = 5.6, 95% confidence interval 1.5-20.6), p=0.009.

Conclusion: PLWH, considered as a whole, are not at a greater risk of MPXV severe disease. However, those with uncontrolled HIV infection, due to lack of effective ART, develop more severe outcomes. Efforts should be done to increase HIV testing and to ensure linkage to HIV care services. In this setting, ART must be immediately started.

905 IMPACT OF HIV INFECTION ON MPXV-RELATED HOSPITALIZATIONS IN BRAZIL


INI-Fiocruz Mpox Study Group

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Background: Late presentation to care remains a major public health problem in Brazil, despite the country’s longstanding commitment to universal access to ART to all PLWH. The COVID-19 pandemic severely hit the country and further impacted the HIV care continuum, with worse disparities observed by gender and sexual orientation. By December 28th, 2022, Brazil reported 10,493 and 14 mpox cases and deaths ranking second global. Although mpox lethality is low, HIV-related immunosuppression may negatively impact mpox outcomes, increasing hospitalizations and fatalities. We aim to describe mpox hospitalization rates and explore the impact of HIV-infection on mpox-related hospitalizations and clinical outcomes.

Methods: Prospective, observational cohort study of individuals with confirmed mpox infection followed at the major mpox referral center in Rio de Janeiro, Brazil. Demographic and clinical data including reasons for hospitalization were systematically collected. Chi-squared or Fisher’s exact tests for qualitative variables and the Moods median test for quantitative variables were used.

Results: From June 12 to December 12, 2022, 402 participants had a laboratory-confirmed mpox diagnosis. Median age was 34 years, 365 (91%) were cisgender men, and 197 (49%) were PLWH. Overall, 39 (10%) participants were hospitalized due to mpox-related causes; 20 (51%) were PLWH. All PLWH with CD4 counts < 200 cells/mm$^3$ required hospitalization. Compared to non-hospitalized PLWH, a higher proportion of hospitalized PLWH had concomitant opportunistic infections (4/20 [20%] vs. 1/177 [0.6%], p < 0.001), were not virologically suppressed (7/20 [35.0%] vs. 22/177 [15.3%, p=0.1) and were not on ART (4/20 [20%] vs. 5/1177 [0.4%], p=0.03). Among all hospitalized participants, PLWH were more frequently hospitalized due to severe proctitis than HIV-negative participants (12/20 [60%] vs. 5/1177 [26.3%, p=0.03], with no differences regarding hospitalizations for pain control (Table). PLWH accounted for all cases of hospitalized individuals who required intensive care support (n=4), had deep tissue involvement (n=3) and had a mpox related death (n=2).

Conclusion: Our findings suggest an association between worse outcomes in the HIV care continuum and mpox-related hospitalizations. Advanced immunosuppression (CD4 < 200) contributed to more severe clinical presentations and death. Public health strategies to mitigate HIV late presentation and the negative impact of the COVID-19 pandemic to the HIV care continuum are urgently needed. Sociodemographic and clinical characteristics of mpox cases according to HIV and hospitalization status

<p>| Table: Sociodemographic and clinical characteristics of mpox cases according to HIV and hospitalization status |
| Hospitalized participant vs. (n=20) |
| Hospitalized participant vs. (n=20) |</p>
<table>
<thead>
<tr>
<th>HIV status</th>
<th>PLWH</th>
<th>HIV-negative</th>
<th>p-value</th>
<th>PLWH</th>
<th>HIV-negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age [years]</td>
<td>34</td>
<td>34</td>
<td>0.99</td>
<td>34</td>
<td>34</td>
<td>0.99</td>
</tr>
<tr>
<td>Males (%)</td>
<td>197 (97%)</td>
<td>927 (97%)</td>
<td>0.78</td>
<td>98 (49%)</td>
<td>258 (76%)</td>
<td>0.01</td>
</tr>
<tr>
<td>PLWH (%)</td>
<td>20 (51%)</td>
<td>127 (36%)</td>
<td>0.03</td>
<td>20 (51%)</td>
<td>127 (36%)</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4 cell count [cells/mm$^3$]</td>
<td>895 [350-3500]</td>
<td>560 [100-3500]</td>
<td>0.01</td>
<td>1205 [350-3500]</td>
<td>560 [100-3500]</td>
<td>0.01</td>
</tr>
<tr>
<td>HIV viral load [log10 copies/mL]</td>
<td>5.6 [4.5-6.8]</td>
<td>5.7 [4.5-6.9]</td>
<td>0.78</td>
<td>5.3 [4.4-6.4]</td>
<td>5.3 [4.2-6.4]</td>
<td>0.99</td>
</tr>
<tr>
<td>Hospitalized due to severe proctitis (%)</td>
<td>12 (60%)</td>
<td>5 (2%)</td>
<td>0.03</td>
<td>12 (60%)</td>
<td>5 (2%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hospitalized due to severe complications (%)</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
<td>0.03</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 No ART: participant switched from care while not on ART; ART: was prescribed or initiated, but patient not on ART at the time of mpox.
CHARACTERISTICS OF THE 2022 MPOX OUTBREAK IN A SOUTHEASTERN US CITY
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Background: In Atlanta, Georgia, the 2022 mpox outbreak disproportionately impacted people who are Black and People with HIV (PWH) compared to other areas. Here, we describe the demographic and clinical manifestations of mpox in Atlanta.

Methods: We performed a retrospective cohort study with manual chart abstractions of 180 individuals who tested positive for mpox at two academic medical centers in Atlanta between 6/1/2022 and 10/7/2022. We used descriptive statistics to summarize demographic and clinical characteristics and Fisher’s exact tests to explore categorical associations between HIV indices, sexual behaviors, and mpox presentations.

Results: Of 180 people diagnosed with mpox, 175 (97.2%) were cisgender men and 5 (2.8%) were transgender women. 160 (88.9%) were Black, 9 (5.0%) Latinx, and 3 (1.7%) White. 152 (84.4%) were PWH. Of 113 PWH with a known HIV viral load (VL), 39 (34.5%) had VL >200 c/µL. Among 107 PWH with a known CD4 count, 15 (14.0%) were < 200, 30 (28.0%) were 201-350, and 62 (57.9%) were >350 c/µL. Of 152 persons with an identified suspected mpox exposure, 143 (94.1%) reported recent sexual contact and 97% reported sex with men. A concurrent syphilis diagnosis occurred in 34 (18.9%) and other sexually transmitted infections occurred in 33 (18.3%). Common initial symptom(s) included: rash (71.7%), fever/chills (43.3%), lymphadenopathy (22.8%), and rectal pain (18.3%). Overall, 77 (42.8%) had mucosal lesions: 38 (21.1%) anorectal, 38 (21.1%) oropharyngeal, 14 (7.6%) urethral, and 2 (1.1%) cutaneous. MPox with CD4 < 200 c/µL were more frequently diagnosed with bacterial superinfection of mpox lesions (p=0.03) and delayed lesion healing >4 weeks (p=0.03). PWH with VL >200 c/µL had more frequent episodes of colitis and GI bleeding (p=0.05). 26 (14.4%) individuals required hospitalization for mpox or mpox complications [24 (92.3%) PWH and 2 (7.6%) people without HIV (PWoH)] and no deaths were observed. People with mucosal involvement (p=0.003) and PWH with VL >200 c/µL (p=0.001) were more frequently hospitalized. Hospital length of stay ≥10 days occurred more frequently in PWH with CD4 < 200 c/µL (p=0.008).

Conclusion: Clinical presentation of mpox in Atlanta was similar to other reports; however, our cohort had a higher burden of HIV co-infection. Severe mpox disease was observed at higher frequency in individuals with uncontrolled HIV, indicating an urgent need to better understand the pathogenesis of HIV-mpox interactions and to develop better prevention and treatment options for PWH.

908 HIV CARE AND PREVENTION CHARACTERISTICS AMONG PERSONS WITH MPOX AND HIV, TEXAS 2022
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Background: A public health emergency was declared for the mpox outbreak by the U.S. Department of Health and Human Services on August 4th, 2022. The Texas Department of State Health Services (DSHS) confirmed the first mpox case in Texas on June 7th, 2022. The objective of this analysis was to describe HIV care and prevention characteristics among persons with mpox and HIV infection in Texas.

Methods: The Texas DSHS conducted a match with the Enhanced HIV/AIDS Reporting System (eHARS) database and the state’s integrated surveillance system to identify persons with mpox and HIV infection. Time from mpox diagnosis to HIV diagnosis and HIV laboratory data was examined to assess HIV care characteristics among persons with mpox and HIV infection. Mpox diagnosis date was estimated using the earliest date of either 1) rash onset, 2) illness onset, 3) positive laboratory result, or 4) health department notification.

Results: As of November 28th, 2022, there were 2,826 confirmed or probable mpox cases in Texas. 1,415 (50%) of persons with mpox had HIV infection. Of those, 55 (3.9%) were diagnosed with HIV infection after or within 30 days of mpox diagnosis, 86 (6.1%) between 30 days to 1 year, 72 (5.1%) between 1 to 2 years, 228 (16.1%) between 2 to 5 years, 418 (29.5%) between 5 to 10 years, and 556 (39.3%) more than 10 years ago. Of those with HIV laboratory testing in the 12 months prior to mpox diagnosis, 1,193 (84.3%) persons had CD4 count data and 1,067 (75.4%) had HIV viral load data. Among those with CD4 counts, 100 (8.4%) had a CD4 count of less than 200 /µL, 313 (26.2%) had CD4 counts between 200–499/µL, 780 (65.4%) had CD4 counts greater than 500/µL. For persons with HIV viral loads, 890 (83.4%) were virally suppressed (< 200 copies/mL), 45 (4.2%) had viral loads between 200–1,000 copies/mL, and 132 (12.4%) had viral loads greater than 1,000 copies/mL.

Conclusion: Prevalence of HIV infection among persons with mpox was high, similar to other findings. The majority of persons with mpox and HIV infection were diagnosed with HIV more than 5 years ago and had HIV laboratory data signifying utilization of HIV care services in the past year. The disproportionate impact of mpox on those with HIV infection reinforces the importance of offering HIV screening testing to persons seeking care for mpox and focusing public health efforts on linkage to HIV care services as needed.

909 MPOX OUTBREAK IN PLWHA AND PREP USERS IN A BRAZILIAN STI CENTER: DIFFERENT CHALLENGES
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Background: Since the beginning of the current mpox outbreak, reports and case series have highlighted a significant distribution in people living with HIV (PLWHA) and also in pre-exposure prophylaxis (PrEP) users. Despite similar
vulnerability behaviors, these populations are distinct from an immunological view. We evaluated the differences in clinical presentation between PLWHA and PrEP users.

**Methods:** We conducted an observational cross-sectional study among adults with suspected Mpox in a public reference institution for the prevention and care of HIV infection and other sexually transmitted infections (STIs) between 6/18/2022 and 9/22/2022. The data were obtained from the Mpox notification database and supplemented by dispensing systems and electronic records. Multivariable analysis was performed using logistic regression models to compare clinical presentation between PLWHA and PrEP users.

**Results:** A total of 394 suspected cases were tested for Mpox RT-PCR, of which 304 (76.4%) were positive. The number of incident cases peaked in July 2022 (Figure 1). PCR-positive patients had a mean age of 33 years, 297 were male and MSM (97%), 247 had anogenital lesions (82.3%), 167 (55%) were PLWH, and 97 (32%) were PrEP users, and no one died. In PLWHA, 85.2% were virologically suppressed patients, while 88.7% have CD4 > 350 cells/mL. The patients with a positive PCR result, compared to patients with a negative result, were more likely to be in the 25–39 age group (OR 2.8; 95% CI 1.1–7.5), have fever (OR 4.7; 2.3–9.7), MSM/transgender women (OR 17.2; 5–56.9), adenomegaly (OR 7.2; 3.8–13.7), multiple vesicular lesions (OR 4.2; 2.1–8.5) and have an STI concurrently (OR 3.3; 1.3–8.6). PLWHA, compared to PrEP users, were more likely to have extragenital involvement (26.3% vs 13.3%; p = 0.016). The two groups did not exhibit any other significant differences in clinical presentation.

**Conclusion:** The Mpox outbreak in Brazil curbed in September, possibly as a result of the strong mobilization of the LGBTQIA+ community. The vast majority of our study participants were PLWHA and PrEP users. PLWA in our study presented more frequently with extragenital involvement than PrEP users, possibly due to a weakened immune response of PLWHA to contain the spread to distant areas. In low-income countries with limited diagnostic resources, the development of an epidemiological and clinical screening prioritizing testing in MSM, young, with fever, adenomegaly and genital lesions, could be a strategy to be implemented.

**MPOX DETECTION RATES (PER 100 ADMISSIONS-MONTH) FROM JUNE TO SEPTEMBER 2022 PER GROUP, CTR SAO PAULO**

911 **MPOX IN AMSTERDAM: CROSS-SECTIONAL STUDY AMONG MSM AT THE CENTRE FOR SEXUAL HEALTH**

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1Public Health Service Amsterdam, Amsterdam, Netherlands, 2Amsterdam University Medical Center, Amsterdam, Netherlands, 3Public Health Department Amsterdam, Amsterdam, Netherlands

**Background:** In May 2022, the first cases of Mpox (Monkeypox) infection were reported in multiple European countries, predominantly among men having sex with men (MSM). Overlap in symptoms of Mpox and other sexually transmitted diseases (STD) made triage for Mpox testing challenging. With this study we aimed to identify characteristics of infection with Mpox among MSM to further strengthen case definitions.

**Methods:** From 20 May 2022 to 15 September 2022 we tested MSM attending the Centre for Sexual Health in Amsterdam, the Netherlands for Mpox (monkeypox) if they met the case definition, using an in-house developed and validated PCR using primers targeting the F3L and G2R Mpox specific genes. We compared socio-demographic and clinical characteristics, sexual behaviour, and STD diagnoses of MSM who tested positive for Mpox, Mpox suspected for Mpox who tested negative and Mpox unsuspected MSM visiting the Center for Sexual Health. In addition, in Mpox positive MSM we compared Cycle threshold (Ct) values of the DNA positive Mpox samples as a proxy for viral load by body condition.

**Results:** Of the 374 MSM tested, 135 (36%) tested positive for Mpox. Comparing the Mpox positive, Mpox negative (n=239) and not tested MSM (n=6,932), the Mpox positive MSM were older (median age respectively 36, 34, and 34 years, p=0.019) and more often lived with HIV (30% versus 16% and 7%, p< 0.001). Furthermore, Mpox positive MSM more often reported receptive anal sex without a condom, more often entered into sexual transmission relationships, and were more often diagnosed with bacterial STD (all p< 0.001), and more often engaged in sexualised drug use (p=0.01). PrEP use was comparable. Systemic symptoms and anogenital lesions were associated with Mpox infection. For Mpox positive
CHARACTERISTICS OF PATIENTS HOSPITALIZED WITH MPOX DURING THE 2022 US OUTBREAK

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Background: During previous mopox outbreaks, severe manifestations of disease and poor outcomes have been reported among people with HIV (PWH), particularly those with AIDS. During the 2022 outbreak, Centers for Disease Control and Prevention (CDC) staff provided clinical consultations for providers caring for patients with mopox and seeking to discuss clinical management or to access therapeutics under the emergency investigative new drug protocol. In this analysis, we sought to characterize the manifestations, outcomes, and HIV care-related considerations of mopox in hospitalized patients to inform care and guide ongoing outbreak response efforts.

Methods: This descriptive analysis assessed characteristics of all hospitalized patients aged >18 years with confirmed mopox infection for whom CDC was consulted between August 10-November 22, 2022. CDC obtained data on patient demographics, clinical course, and outcomes during consultation with health departments or providers.

Results: Of 103 patients hospitalized with mopox infection, 100 (97%) were male, and the median age was 34.5 years (range = 20–61 years). Most patients were non-Hispanic Black (60%), and 22 (21%) were experiencing homelessness. Ninety (97%) had HIV infection; among these patients, 49 (50%) of 94 with a known CD4 count had < 50 CD4 cells/mm3. Of patients with HIV infection, 14 (16%) were receiving antiretroviral therapy (ART) before mopox diagnosis. Manifestations included dermatologic involvement (96, 93%), severe mucosal lesions (76, 74%), and involvement of the lungs (21, 20%), eyes (23, 22%), gastrointestinal system (5, 5%), and brain or spinal cord (four, 7%). Twenty-three (22%) patients received ICU-level care and 20 (19%) died.

Conclusion: Mopox infection in the current U.S. outbreak has been associated with severe morbidity and mortality, particularly among persons with AIDS. The disproportionate burden of severe mopox among persons of color and persons experiencing homelessness echoes inequities seen in the continuum of care for PWH. Providers should test sexually active patients with suspected mopox infection for HIV and other sexually-transmitted infections as indicated at the time of mopox testing. Engaging all PWH in care remains a critical public health priority, with additional efforts in HIV outreach and care retention needed to reduce the population at risk for severe mopox.
914 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF MPOX WITHIN A NEW YORK CITY HEALTH SYSTEM

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Background: Since the global mpox outbreak began in Spring 2022, it has disproportionately affected gay and bisexual individuals. Mpox can cause serious illness, including painful mucosal lesions, superinfections, and ocular disease. It presents in hospitalized patients and those with HIV/AIDS and other immunocompromising conditions is not yet well described, nor is the efficacy of therapies such as tecovirimat. Here we describe the clinical characteristics and course of individuals diagnosed with mpox in a large New York City health system who were prescribed tecovirimat.

Methods: This retrospective study described clinical features of persons with mpox and the clinical outcomes amongst those who received tecovirimat. Data was obtained via chart review of patients prescribed tecovirimat in the Mount Sinai Health System in NYC during 7/1/2022 - 10/1/2022.

Results: 129 people diagnosed with mpox were prescribed tecovirimat between 7/1/2022 and 10/1/2022. The median patient age was 37 years, 95% were men, and 9% identified as gay or bisexual. 25% of the cohort identified as Hispanic/Latina, 46% White, 35% Black, 9% Asian, and 12% reported/other. 62% had HIV, 49% of these with undetectable HIV viral loads and 11% with a CD4 cell count of < 200 cells/mL. Nearly all (96%) presented with rash, while 71% had anogenital lesions, 22% with oral mucosal lesions, and 4% had ocular involvement. Presenting symptoms included non-specific pain (76%), painful defecation (55%), and odynophagia (35%). Common systemic features included fever/chills (49%), lymphadenopathy (35%), fatigue (26%), and myalgias (26%). Of those receiving tecovirimat with completed follow-up (N=85; 66%), 11% had anogenital lesions, 22% with oral mucosal lesions, and 4% had ocular involvement. Presenting symptoms included non-specific pain (76%), painful defecation (55%), and odynophagia (35%). Common systemic features included fever/chills (49%), lymphadenopathy (35%), fatigue (26%), and myalgias (26%). Of those receiving tecovirimat with completed follow-up (N=85; 66%), 47% had recovery of lesions by time of post-treatment assessment (median of 8 days after finishing therapy). The median time for complete lesion resolution on tecovirimat was 10 days. Tecovirimat was well tolerated; there were no severe adverse effects attributable to the therapy. Hospitalized patients (n=16; 12% of total) were primarily admitted for superimposed bacterial infections (62%), with a median hospital stay of 4 days. 69% (n=11) of hospitalized patients had HIV; of these, 5 patients were severely immunocompromised, either due to AIDS or an additional immunocompromising condition.

Conclusion: In a diverse cohort of mpox patients, treatment with tecovirimat was well tolerated and associated with minimal adverse effects. The majority of hospitalizations occurred in patients with underlying immunocompromising conditions.

915 MPOX INFECTION IN WOMEN: A CASE SERIES FROM BRAZIL

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The WHO declared thatcko-mpox as a global emergency in May 2022. Since the global mpox outbreak began in Spring 2022, it has disproportionately affected gay and bisexual individuals. Mpox can cause serious illness, including painful mucosal lesions, superinfections, and ocular disease. It presents in hospitalized patients and those with HIV/AIDS and other immunocompromising conditions is not yet well described, nor is the efficacy of therapies such as tecovirimat. Here we describe the clinical characteristics and course of individuals diagnosed with mpox in a large New York City health system who were prescribed tecovirimat.

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Conclusion: In a diverse cohort of mpox patients, treatment with tecovirimat was well tolerated and associated with minimal adverse effects. The majority of hospitalizations occurred in patients with underlying immunocompromising conditions.

916 SARS-CoV-2 SEROPREVALENCE AMONG UGANDAN BLOOD DONORS: 2019-2022

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Background: COVID-19 in Africa was less severe with fewer reported cases, hospitalizations and deaths compared to other continents. However, the lack of adequate surveillance systems in Africa makes estimating the burden of infection challenging. Serosurveillance can aid in determining the frequency of infection within this population. This study is aimed to estimate SARS-CoV-2 seroprevalence, describe the SARS-CoV-2 antibody (Ab) levels, and examine associations of seroreactivity among Ugandan blood donors.

Methods: Samples were obtained from the Mirasol Evaluation of Reduction in Infections Trial (MERIT), a randomized, double blind, controlled clinical trial evaluating transfusion transmitted infections. MERIT blood donor samples (n=3,517) were collected from Kampala, Uganda between October 2019 to April 2022. Additional blood donor samples (n=1,876) were collected from across the country between November-December 2021. Samples were tested for Ab to SARS-CoV-2 nucleocapsid (N) and spike (S) using an electrochemiluminescence immunoassay assay (Meso Scale Diagnostics, Gaithersburg, MD) per manufacturer’s protocol. Samples seroreactive to both N and S Ab were considered Ab positive to SARS-CoV-2. Seroprevalence among MERIT donors was estimated within each quarter. Factors associated with seroreactivity from November-December 2021 were assessed by chi-square test.

Results: SARS-CoV-2 seroprevalence increased from < 2.0% in October 2019- June 2020 to 82.5% in January-April 2022. Three distinct peaks in seroreactivity were seen in October-November 2020, July-August 2021, and January-April 2022 (see Figure). Among seroreactive donors, median N Ab levels increased...
9-fold and median S Ab 19-fold over the study period. In November-December 2021, SARS-CoV-2 seroprevalence was higher among donors from Kampala (58.8%) compared to more rural regions of Hoima (47.7%), Jinja (47.9%), and Masaka (54.4%; p<0.007); S seroprevalence was lower among HIV+ donors (58.8% vs. 84.9%; p=0.009).

**Conclusion:** Blood donors in Uganda showed high prevalence of Ab to SARS-CoV-2 by March of 2022, indicating that the infection levels were similar to many other regions of the globe. Higher seroprevalence was observed in the capital compared to more rural areas in Uganda. Further, increasingly high antibody levels among seropositive donors may indicate repeat infections. The lower COVID-19 morbidity and mortality was not due to a lack of exposure of the virus, but other factors yet to be determined.

SARS-CoV-2 seroprevalence among Ugandan blood donors and COVID-19 vaccination in Uganda from 2019 to 2022

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917 COVID-19 SEROPREVALENCE IN VACCINE-NAIVE POPULATIONS: DRC, GUINEA, LIBERIA, AND MALI

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InVITE Study Team

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**Background:** As part of an international multi-country study on COVID-19 vaccine immunogenicity (InVITE, NCT05096091), we sought to characterize baseline anti-NSpike protein (N) and anti-Spike (S) seropositivity by country and by self-report of prior positive SARS-CoV-2 test result.

**Methods:** 3063 vaccine-naive individuals from the InVITE study cohort, who received a COVID-19 vaccine as part of their country’s national immunization programs at participating sites in Democratic Republic of Congo (DRC), Guinea, Liberia and Mali, were enrolled between August 2021 and February 2022. Demographic and baseline characteristics were collected at study enrollment. Blood was collected at baseline prior to initiation of the vaccine regimen. SARS-CoV-2 anti-S and anti-N antibody levels were measured using Quanterix anti-S IgG semi-quantitative antibody and BioRad Platelia SARS-CoV-2 anti-S antibody and anti-N antibody levels were measured using serological assays that detect adaptive immune responses to SARS-CoV-2 spike protein. Modified Poisson regression models were used to calculate prevalence rate ratios (PRR) and 95% confidence intervals (CI) to identify sociodemographic and clinical risk factors.

**Results:** Plasma samples from 979 PROMOTE mothers and 1332 children were analysed. We found no significant differences in baseline characteristics between participants testing positive (+) and negative (-) for SARS-CoV-2 Ab. Overall maternal SARS-CoV-2 seroprevalence was 57.6% (95%CI: 54.5-60.7) and 39.3% (95%CI: 36.7-41.9) for infants. The earliest + result was detected from a sample collected on 09/2019, in Malawi. Factors significantly associated with SARS-CoV-2 seropositivity were country of origin (reference Uganda, aPRR 1.45, 95%CI: 1.24-1.69) and non-breastfeeding mother (aPRR=1.22, 95%CI: 1.02-1.48). Children above 5 years had a 10% increased risk of SARS-CoV-2 seropositivity (aPRR=1.10, 95%CI: 0.90-1.34) when compared to younger children. We found no statistically significant association with sanitation, household density, distance to clinic, maternal employment, ART regimen or viral load. Mother-infant SARS-CoV-2 serostatuses were discordant in 373/865 (43.1%) families tested: mothers+children- in 51.2%; mothers-/children+ in 12%; child+/sibling- concordance was 21.4%.

**Conclusion:** These SARS-CoV-2 seroprevalence data indicate that by late 2021, about half of mothers and about a third of children in a cohort of HIV-affected families in eastern/southern Africa had been infected with SARS-CoV-2. Breastfeeding was protective for mothers, likely because of the need to stay home for young children. Discordant results between children within same families underscores the need to further understand transmission dynamics within households.

919 SARS-CoV-2 INFECTION AMONG PERSONS WHO INJECT DRUGS AND THEIR PARTNERS IN KENYA

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**Background:** Despite increased social vulnerability and barriers to care, there has been a paucity of data on SARS-CoV-2 incidence among key populations in sub-Saharan Africa. We seek to characterize active infections and define transmission dynamics of SARS-CoV-2 among people who inject drugs (PWID) and their sexual and injecting partners from Nairobi and the coastal region in Kenya.

**Methods:** This was a nested cross-sectional study of SARS-CoV-2 infection from April to July 2021 within a cohort study of assisted partner services for PWID in Kenya. A total of 1000 PWID and their partners (500 living with and 500 living without HIV) were recruited for SARS-CoV-2 antibody testing, of whom 440 were randomly selected to provide self-collected nasal swabs for real-time...
SARS-CoV-2 SEROPREVALENT AMONG PEOPLE WHO INJECT DRUGS IN BALTIMORE

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Background: People who inject drugs (PWID) may be at a greater risk of SARS-CoV-2 infection and COVID-19 due to socio-structural inequities, high-risk behaviors and comorbidities; however, PWID have been underrepresented in case-based surveillance due to lower access to testing. We characterize temporal trends and correlates of SARS-CoV-2 seroprevalence among a community-based sample of current and former PWID.

Methods: A cross-sectional study was conducted among participants in the AIDS Linked to the IntraVenous Experience (ALIVE) study—a community-based cohort of adults with a history of injection drug use in Baltimore, Maryland. Participants’ first serum sample collected at routine study visits between December 2020 and July 2022 was assayed for antibodies to the nucleocapsid (N) (past infection) and spike-1 (S) (past infection and/or vaccination) proteins using the MSD V-Plex Panel 2 IgG SARS-CoV-2 assay. For each correlate, we estimated adjusted prevalence ratios (PR) via separate Poisson regression models adjusted for calendar time, age, sex and race.

Results: Of 561 participants, the median age was 59 years (range=28-77), 35% were female, 64% were Black, 36% were living with HIV (97% on ART), and 55% had received ≥1 COVID-19 vaccine dose. Overall, anti-N and anti-S prevalence was 26% and 63%, respectively. Prevalence of anti-N increased from 23% to 40% between December 2020-May 2021 and December 2021-July 2022, with greater increases in the prevalence of anti-S from 34% to 86% over the same period (Figure). Being employed (PR=1.53 [95%CI=1.11-2.11]) and never being married (PR=1.40 [0.99-1.99]) were associated with a higher prevalence of anti-N, while female sex (PR=0.75 [0.55-1.02]) and a history of cancer (PR=0.40 [0.17-0.91]) were associated with a lower prevalence of anti-N. Younger age, female sex (PR=0.90 [0.80-1.02]), and homelessness (PR=0.78 [0.60-0.99]) were associated with a lower prevalence of anti-S. Although HIV infection was not associated with anti-N, it was associated with a higher prevalence of anti-S (PR=1.13 [1.02-1.27]). Substance use was not associated with anti-N or anti-S.

Conclusion: Anti-N and anti-S levels increased over time, suggesting cumulative increases in SARS-CoV-2 incidence of infection and vaccination among PWID; however, disparities in seroprevalence remain. Younger and female PWID and those experiencing homelessness were less likely to be anti-S positive, suggesting programs should aim to improve vaccination coverage in such vulnerable populations.

Temporal trends and correlates of SARS-CoV-2 antibodies among people who inject drugs
infection and hospitalization. Younger age groups tended to have much higher rates of natural or hybrid immunity with 96% of 0–17-year-olds and 83% of 18–49-year-olds protected due to past infections. Overall, the population-level immunity against the Omicron variant reduced risk of infection by 59% (95% Credible Interval 54% – 62%) and risk of hospitalization by 79% (95% CI 77% – 81%) in Washington and 62% (95% CI 57% – 66%) and 83% (95% CI 82% – 85%), respectively, in Oregon. There was similar population-level protection against Delta at the start of the Omicron wave in early December 2021, which reduced risk of infection by 60% (95% CI 56% – 63%) and risk of hospitalization 79% (95% CI 78% – 80%) in Washington and 66% (95% CI 63% – 70%) and 82% (81% – 83%), respectively, in Oregon.

**Conclusion:** Very large waves of new infections throughout 2021 and early 2022, in addition to high levels of vaccination and boosting among the older age groups in Washington and Oregon have greatly reduced population susceptibility to currently circulating strains. However even very high population immunity has allowed for emergence of novel variants that escape existing immunity, highlighting the need for continued develop of new variant-specific boosters.
**924 RETROSPECTIVE ASSESSMENT OF THE ACCURACY OF MPXV CASE PROJECTIONS IN NEW YORK CITY**

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**Background:** In mid-2022, New York City (NYC) became the epicenter of the US mpox epidemic. Health authorities were in need of forecasts to anticipate the timing and magnitude of the outbreak. We provided mathematical model-based projections with methodologies that evolved alongside the epidemic. Here, we retrospectively evaluate our mpox case projections and reflect on potential reasons for accuracies and inaccuracies.

**Methods:** Early in the outbreak (July 1 – 15, 2022), when the size of the at-risk population was unknown, we performed short-term (2-week) forecasting using exponential regression. Once epidemic growth was no longer exponential (July 15 – Aug, 9), we consulted with the NYC Department of Health and Mental Hygiene regarding populations most-at-risk of mpox based on available epidemiological data. Modelers and epidemiologists collaboratively developed an estimate of 70,180 people at risk, informed by estimates of LGBTQ adults with male sex at birth who had 2+ partners in the last 3 months. We combined this with NYC case count data, NYC vaccination data, and global mpox natural history data to develop a Susceptible-Exposed-Infected-Recovered (SEIR) model, taking into account immunity accrued through vaccination and prior exposure, for longer-term forecasting.

**Results:** Initial exponential forecasts of the NYC mpox outbreak were only accurate for very short-term predictions (<2 weeks) (Figure, top panel). Forecasts were more accurate after 1 week (mean absolute error: 83.8 cases/ wk) than after 2 weeks (mean absolute error: 351.4 cases/wk). In contrast, the SEIR model accurately predicted the decline in cases through the end of Sept. 2022, when cases fell to <70/wk. Over the period from Aug. 10 to Sept. 24 2022, the mean absolute error of the SEIR-based projection was 8.2 cases per week (Figure, bottom panel).

**Conclusion:** Model-based NYC mpox projections provided only short-term accuracy in the early epidemic, but long-term accuracy once the epidemic exited exponential growth and an SEIR model was developed. Cumulative cases and vaccinations when exiting exponential growth, and the epidemiology of those most-at-risk, provided evidence for the likely size of the most-at-risk population — a crucial input for an accurate SEIR model. The ability of the SEIR model to accurately forecast mpox cases was in part attributable to lack of vaccine or immune escape by mpox, in contrast to more rapidly-evolving viruses such as SARS-CoV-2.

**Weekly Mpox Cases and Model Projections**

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**925 MODELLING THE MPXV EPIDEMIC IN THE UK OVER MAY-AUGUST 2022**

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*Presented at CROI by a nonauthor colleague

**Background:** The monkeypox epidemic in the UK began in May 2022. Unexpectedly and rapidly, rates of new cases declined during August 2022. Identifying the causes of this decline requires accurate estimates of the time-varying epidemic growth rate, which in turn depend upon the delay between symptom onset to healthcare presentation. In this paper, we utilise modelling and monkeypox data from the UK to quantify the changes in this delay and their effect on estimating the epidemic growth rate of monkeypox over the period May-August 2022 in the UK.

**Methods:** We developed a custom nowcasting Bayesian method which incorporates time-varying delays (EpiLine), simulating the growth rate of symptomatic cases and the parameters of delay distributions following Gaussian processes. We applied our model to the monkeypox data from the UK, sampling the posterior distribution of all parameters using Markov Chain Monte Carlo methods, to quantify the delay between monkeypox symptom onset to healthcare presentation and the growth rate over the study period.

**Results:** Our results suggest that the delay between symptom onset and healthcare presentation for monkeypox in the UK decreased from an average of 22 days in early May 2022 to 10 days by early June and 7 days in August 2022. When we account for these dynamic delays, the time-varying growth rate declined gradually in the UK over the May-September 2022 period. Not accounting for varying time delays would have incorrectly characterized the growth rate by a sharp increase followed by a rapid decline in monkeypox cases.

**Conclusion:** Our results highlight the importance of correctly quantifying the delay between symptom onset to healthcare presentation when characterizing the epidemic growth of monkeypox in the UK. We show that reducing the delay in accessing healthcare is crucial as shorter delays can prevent onwards transmission, and allows prompt use of antivirals post infection. Hence, our study highlights the importance and need for public health agencies to focus on reducing delays from symptom onset to healthcare presentation early in an outbreak and when tailoring the optimal policy response.
WHAT IMPACT WILL WORSENING AIR POLLUTION HAVE ON THE BURDEN OF COVID-19?
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Background: Evidence suggests association between long-term exposure to air pollutants and increased risk of becoming infected with SARS-CoV-2, the causative agent of COVID-19, and increased severity of COVID-19. However, it remains unclear whether breathing more polluted air over many years affects susceptibility to infection or only affects disease severity, with uncertainty around the intensity of these associations. It has been estimated that anthropogenic emissions have contributed to over 10% of the over 660 million cases of SARS-CoV-2 and the over 7.5 million COVID-19 deaths reported worldwide over the course of the pandemic. Furthermore, as the world continues to warm and if air pollution levels increase, then so might the burden of respiratory infectious disease, including COVID-19.

Methods: Here we explore the potential impact of long-term exposure to increasing levels of particulate matter 2.5 microns or less in diameter (PM2.5) (+1 to +5 μg/m3) assuming an association on either (1) SARS-CoV-2 susceptibility or (2) COVID-19 disease severity by projecting SARS-CoV-2 infections and COVID-19-related hospital admissions over a two-year period. Simulations were conducted using a SARS-CoV-2 transmission model in a global setting capturing age and comorbidity risk, considering seasonality, emerging variants, and vaccination and treatment options. We model linear, log, and log10 relationships between these associations.

Results: We show that if long-term exposure to higher levels of air pollution only affects COVID-19 severity, then as expected, the projected number of COVID-19-related hospitalisations would proportionally increase. However, if exposure directly affects the susceptibility of becoming infected, then while infections would be higher, hospitalizations would also be even higher due to the potential for onward transmission. This aligns with associations between air pollution and other respiratory infections and their associated health outcomes.

Conclusion: The anticipated additional impact air pollution is having on the public health burden of respiratory infectious disease, like COVID-19, should be considered in strategic action plans to mitigate and adapt to changing levels of air pollution. It is important to better understand at which point air pollution affects SARS-CoV-2 infection acquisition through to disease progression, to enable improved protection and to better support those most vulnerable.

Submitted by Sherrie L. Kelly, Switzerland.
**HIGH PERFORMANCE OF BLOOD-BASED HIV SELF-TESTS AT PRIVATE PHARMACIES IN KENYA**

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**Background:** HIV self-testing (HIVST) has the potential to support daily oral pre-exposure prophylaxis (PrEP) delivery in new community-based settings, but guidelines have not approved HIVST for PrEP dispensing. In Kenya, pharmacy providers are permitted to deliver HIVST, but to deliver rapid diagnostic testing (RDT), a certificate is required that few have. We sought to understand the performance of provider-delivered blood-based (BB) HIVST compared to RDT, the standard of care for PrEP delivery, at private pharmacies in Kenya to inform the possible use of HIVST for PrEP scale-up.

**Methods:** At 20 pharmacies in Kisumu County, we trained pharmacy providers (pharmacist & pharmaceutical technologists) on BB HIVST use and client assistance (if requested). We recruited pharmacy clients purchasing sexual and reproductive health-related products (e.g., condoms) and enrolled those ≥18 years with behaviors associated with HIV risk (e.g., partners of unknown HIV status). Enrolled clients received BB HIVST with associated provider counseling, followed by RDT by a certified HIV testing services (HTS) counselor. Pharmacy clients, providers, and HTS counselors independently interpreted the HIVST results prior to the RDT results (interpreted only by the HTS counselor). We calculated the sensitivity and specificity for BB HIVST compared to RDT.

**Results:** Between March and June 2022, we screened 1691 clients and enrolled 1500; 64% (n=954) were female and the median age was 26 years (IQR 22-31). We additionally enrolled 40 providers; 42% (n=17) were pharmacy owners and the median time in the profession was 6 years (IQR 4-10). The majority (79%, n=1190) of clients requested provider assistance with HIVST, and providers reported spending a median of 20 minutes (IQR 15-43) with each HIVST client. Compared to RDT, the performance of provider-delivered BB HIVST was high when interpreted by clients (sensitivity 98.8%, 95%CI 98.0%-99.3%; specificity 93.8%, 95%CI 84.8%-98.3%), providers (sensitivity 98.5%, 95%CI 97.8%-99.1%; specificity 96.9%, 95%CI 89.2%-99.6%), and HTS counselors (sensitivity 98.5%, 95%CI 97.7%-99.0%; specificity 98.4%, 95%CI 91.6%-100.0%), Fig. 1.

**Conclusion:** When compared to the national HIV testing algorithm, the performance of provider-delivered BB HIVST at private pharmacies in Kenya was high. These findings suggest that BB HIVST may be considered as a testing option to support PrEP initiation and continuation at private pharmacies and potentially other community-based delivery settings.

![Diagram of performance comparison between RDT and HIVST in Kenya](image-url)

**REMOVAL OF A SUBSIDY FOR HIV SELF-TESTING KITS REDUCES ONLINE KIT SALES IN KENYA**

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**Background:** HIV self-testing (HIVST) can enable individuals to learn their HIV status confidentially, without fear of stigma, which may result in an earlier linkage to prevention or treatment interventions. To help increase HIVST access, international donors and nongovernmental organizations partnered to subsidize HIVST kits in 50 low-income countries. In Kenya, this subsidy started in 2019 and ended in 2021. To understand the impact of this subsidy removal on online HIVST kit sales in Kenya, we used controlled interrupted time series (cITS) methods, a quasi-experimental approach.

**Methods:** For our analysis, we used bi-weekly sales data from MYDAWA, the first licensed fully online pharmacy in Kenya. During the subsidy period, both oral-fluid (OF) and blood-based (BB) HIVST kits were available via MYDAWA at 250 KES (~$2.3 USD) per kit. On March 3, 2022, MYDAWA ran out of subsidized OF HIVST kits and the price for these kits rose to 470 KES (~$4.3 USD); on May 25, 2022, the price of BB HIVST kits rose to 760 KES (~$6.9 USD) for the same reason.

We conducted a cITS analysis to understand the impact of the subsidy removal on online OF and BB HIVST kit sales via MYDAWA, using sales of an emergency contraceptive product as a control.

**Results:** From June 2021 to September 2022, we had 32 bi-weekly time units of online sales data for HIVST kits and related products. For OF HIVST kits, we had 19 time units in the pre-subsidy and 13 time units in the post-subsidy period; for BB HIVST kits, we had 21 time units in the pre-subsidy and 11 time units in the post-subsidy period. When the subsidy was removed for OF HIVST kits, this reduced online bi-weekly sales by 273%, with 82 fewer kits (95% CI 47-117) sold bi-weekly compared to the control, Fig. 1. Then, when the subsidy was removed for BB HIVST kits, this reduced bi-weekly online sales by 690%, with 69 fewer kits (95% CI 49-89) sold bi-weekly compared to the control.

**Conclusion:** Removal of a subsidy for HIVST kits significantly decreased the online sale of both OF and BB HIVST kits in Kenya, resulting in potential missed opportunities for early HIV detection and linkage to prevention and treatment services. This evidence suggests that subsidies are effective at increasing the demand for HIVST and should be considered to increase HIV testing in Kenya and similar settings.

![Graph showing impact of subsidy removal on OF and BB HIVST kit sales](image-url)
931 VIRTUAL SUPPORT IMPROVES CLIENT EXPERIENCES WITH AN ONLINE HIV SELF-TESTING SERVICE
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Background: HIV self-testing (HIVST) has the promise to efficiently reach vulnerable populations who are hesitant or unable to visit healthcare locations for testing, especially as more affordable tests become available. We implemented a novel web-based HIVST service in India, which reached many who had never tested before and with high positivity. To understand how to scale HIVST and maximize efficiency, we assessed barriers and facilitators of the experience through a brief online survey.

Methods: The HIVST web-based service was launched in July 2021. HIVST kits were couriered to clients or picked up at select locations. Virtual counselors (VCs) were available to clients for pre/post-test counseling and assistance with using the kits, including interpreting and uploading results to the website, and linkage to appropriate services. For clients who agreed to be contacted later about their experiences, an automated WhatsApp message with a link to a web-based self-administered survey was sent. The survey included basic demographics and their experiences with ordering, taking the test, reporting results, and interactions with VCs.

Results: As of August 2022, 3014 clients ordered an HIVST, of whom 87% received the kit, 82% uploaded their result, and 5% screened positive. Seventy-four percent were male, 64% 18-30 years of age, and 45% of kits were sent via courier. From October 2021-August 2022, 305 clients completed the survey; 87% of respondents were men and 81% had kits couriered. Nearly a quarter had ordered more than one kit and 5% had a verified positive HIVST result. A majority (87%) reported that it was easy using the website for ordering and receiving the kit. While most (85%) reported understanding the kit instructions and confidence about what to do with the result, the majority (81%) also reported receiving help completing the test - 76% from VCs and 14% from a friend. Most (88%) would recommend HIVST to others. Compared to those that did not get help from VCs, clients helped by VCs were more likely to understand kit instructions, complete the test, be confident in knowing what to do based on result, report the result, and recommend HIVST to others (Table). Conclusion: Clients were able to easily order, receive, and use HIVST kits from a web-based service. However, it is notable that most had interaction with a VC who facilitated the process. Virtual support by trained counselors could improve efficiency, uptake, and linkage of both virtual and in-person HIV prevention and treatment services.

932 PEOPLE WHO INJECT DRUGS’ WILLINGNESS TO USE AND DELIVER HIV SELF-TEST KITS TO PEERS
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Background: HIV self-testing (HIVST) kits, including their secondary distribution in social networks, promote HIV testing but remain understudied among people who inject drugs (PWID). To inform efforts to address barriers to standard facility-based HIV testing among PWID, we assessed willingness to use and distribute HIVST kits among PWID along the San Diego-Tijuana border.

Methods: From 2020-2021, 612 PWID in San Diego, USA, and Tijuana, Mexico, underwent HIV testing and completed interviewer-administered surveys. Modified Poisson regression examined associations between willingness to use and distribute HIVST kits and socio-demographics, sexual and substance use behaviors, HIV testing history, and social network characteristics.

Results: Among 539 HIV-negative PWID, median age was 43 years (IQR=35-52), 75% were male, 72% Latinx, 75% had ever tested for HIV, and mean social network size was 3.2 members. Overall, 81% were willing to use HIVST kits themselves, and 81% were willing to distribute them in their networks (79% to sex partners; 75% to drug use partners). Willingness to use HIVST kits oneself was associated with willingness to distribute them (prevalence ratio [PR]=8.4, 95% confidence interval [CI]: 4.9-14.2). At the individual level, prior HIV testing was positively associated with willingness to use (PR=1.3, 95% CI: 1.1-1.4) and distribute (PR=1.3, 95% CI: 1.2-1.5) HIVST kits, while perceiving oneself to be at higher HIV risk than other PWID was negatively associated with willingness to use (PR=0.8, 95% CI: 0.7-0.9) and distribute (PR=0.8, 95% CI: 0.7-0.9) HIVST kits. At the network level, willingness to distribute HIVST kits was positively associated with network size (PR=1.05 per network member, 95% CI: 1.01-1.08) and greater proportions of one’s network being homeless (PR=1.6, 95% CI: 1.4-1.9), detained/arrested (PR=1.7, 95% CI: 1.2-2.0), and using heroin (PR=1.3, 95% CI: 1.1-1.6) and cocaine (PR=1.5, 95% CI: 1.3-1.7). Willingness to distribute HIVST kits was lower among those whose networks consisted of a greater proportion of persons they consider “very close” to them (PR=0.8, 95% CI: 0.7-0.9).

Conclusion: We found high levels of willingness to use and distribute HIVST kits among PWID, and high potential for secondary distribution to increase HIV testing among PWID who face the greatest barriers to standard testing. Efforts to bolster HIV knowledge and address fears of stigma from close peers may enhance the impact of HIVST among PWID.

933 OVERCOMING ACCESS BARRIERS TO VIRAL SUPPRESSION TESTING BY SELF-MICROCOLLECTED BLOOD
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Background: Structural and socio-behavioral barriers to clinic access for continuum of HIV care are major hurdles to medication adherence in areas of lower socioeconomic status. An important indicator of well-being for persons with HIV (PWH) is sustained engagement in care to support viral suppression (VS). Remote VS testing may be supported by self-collected blood in microcollection tubes (MCT), which has benefits over dried blood spots (DBS).

Methods: We developed a kit with instructions and procedure for remote VS testing using fingerstick blood in EDTA- preservative MCT (Microtainer®) and ambient temperature shipping with temperature monitoring. Separated plasma was used for semi-quantitative RNA testing in a 1.7 dilution protocol that, compared to DBS testing, allows for greater sensitivity (LOQ 210 cp/mL, 99% quantified at 350 cp/mL), requires less sample manipulation, and alleviates DNA contamination. MCTs from 90 participants from various HIV study cohorts willing to self-collect and mail specimens were assessed for allowable shipping time (<4 days), transit temperature (<35°C) and plasma volume (>100 µL). Samples that met the criteria were HIV-1 RNA tested. For 28 participants, a same-day venipuncture sample was obtained for comparison to traditional viral load (VL).

Results: Of the 90 mailed MCTs, 12 (13%) were rejected on receipt: eight were below volume, three exceeded the temperature limit and one exceeded the shipping period. Four of six participants who obtained another self-collect kit after an unsuccessful fingerstick collection were able to obtain and mail an MCT sample. In total, 81% of persons who attempted self-collection provided a suitable specimen. Seventy-two persons documented to be on PrEP or who were virally suppressed had no viral target detected in the self-collected samples. The six individuals with a quantitated venipuncture VL also had RNA quantitated in MCT (VL range 273 -11,918 cp/mL). Results from the MCT were within 0.31 log10 of the VL provided by venipuncture. Hemolyzed specimens were included as hemolysis did not affect test results.

Conclusion: Untrained individuals were able self-collect and ship sufficient blood volume to support remote viral suppression testing. Gained experience

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934 RACIAL/ETHNIC DISPARITIES IN HIV TESTING EXPERIENCE BEFORE HIV DIAGNOSIS: 2014–2019

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Background: In 2019, Black/African American and Hispanic/Latino persons accounted for 13% and 18% of the US population, but 42% and 29% of HIV diagnoses. This analysis examines HIV testing prior to HIV diagnosis by race/ethnicity to describe trends in testing experience and identify which groups could benefit from increased testing and linkage to care.

Methods: We used National HIV Surveillance System data in 21 jurisdictions to assess trends (estimated annual percent change (EAPC) and 95%CI) in HIV testing patterns among Black/African American, Hispanic/Latino and White persons aged ≥13 years with HIV infection diagnosed 2014–2019 by sex, age and transmission category.

Results: For all races/ethnicities, the percentage who ever had a previous negative HIV test before diagnosed HIV infection decreased from 2014 to 2019 [67% to 58%;EAPC=-2.6;95%CI=-3.0,-2.2]. This decrease was highest in White [70% to 61%;EAPC=-2.9;95%CI=-3.8,-2.0], followed by Hispanic/Latino [64% to 57%;EAPC=-2.7;95%CI=-3.5,-1.9] and Black/African American [66% to 58%;EAPC=-2.5;95%CI=-3.2,-1.8] persons. Significant decreases occurred for males and females with infection attributed to male-to-male sexual contact (70% to 62%;EAPC=-2.3;95%CI=-3.1,-1.4), male heterosexual contact (54% to 44%;EAPC=-3.8;95%CI=-4.6,-1.4), and female heterosexual contact (62% to 52%;EAPC=-3.7;95%CI=-5.2,-2.2). For Hispanic/Latino persons, significant decreases occurred for those with infection attributed to male-to-male sexual contact (67% to 59%;EAPC=-3.0;95%CI=-3.9,-2.1) and male heterosexual contact (58% to 51%;EAPC=-3.1;95%CI=-5.8,-0.3). For White persons, significant decreases occurred only for those with infection attributed to male-to-male sexual contact (73% to 63%;EAPC=-3.0;95%CI=-4.0,-2.0).

Conclusion: Further research is needed to determine whether decreases in HIV testing before diagnosis among persons in these racial/ethnic groups are associated with trends in uptake of HIV testing and prevention strategies in the general population. Annual HIV testing and tailored prevention strategies should be promoted among all persons with HIV risk factors to increase early detection and linkage for improving HIV care outcomes and reducing risk for HIV transmission.

935 CDC-FUNDED HIV TESTING AND UNDIAGNOSED HIV INFECTION IN ENDING THE HIV EPIDEMIC AREAS

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Background: HIV testing is a key component to diagnosing individuals and linking them to care. However, approximately 14% of persons with HIV (PWH) are unaware of their HIV status. To help reach the goals of the Ending the HIV Epidemic in the U.S. (EHE) initiative, it is important to understand the relationship between HIV testing rates and rates of undiagnosed HIV infection, particularly in areas with high HIV prevalence.

Methods: Using 2019 data from the National HIV Surveillance System and the National HIV Prevention Program Monitoring & Evaluation system, we calculated estimated undiagnosed HIV infection per capita (i.e., rate of undiagnosed HIV infections per 100,000 population) and CDC-funded HIV tests per capita (i.e., rate of CDC-funded HIV tests per 1,000 population) in Phase 1 EHE areas. We assessed the association between the two rates using Spearman’s rank correlation. We also calculated a rank difference between the two rates for each EHE area to help understand which areas have greater unfulfilled needs for HIV testing.

Results: CDC-funded HIV tests per capita was positively associated with estimated undiagnosed HIV infection per capita (r=0.59, p<0.001). The EHE areas with the largest rank differences (i.e., higher undiagnosed HIV infection per capita and lower CDC-funded HIV tests per capita) were Miami-Dade County, FL; Prince George’s County, MD; Hudson County, NJ; Bronx County, NY; and Hamilton County, OH. The EHE areas with the smallest rank differences were San Francisco County, CA; Tarrant County, TX; Suffolk County, MA; Missouri; and Alabama.

Conclusion: There is a significant association overall between CDC-funded HIV tests per capita and estimated undiagnosed HIV infection per capita, indicating that—in general—CDC-funded HIV testing is being conducted in areas with the greatest needs. However, some EHE areas had large discrepancies between CDC-funded HIV tests per capita and estimated undiagnosed HIV infection per capita. These aforementioned areas could use this information to identify barriers to their HIV testing services and improve or expand upon their HIV testing programs to help ensure that all PWH in their jurisdictions are diagnosed and linked to HIV medical care, prevention, and supportive services.

CDC-Funded HIV Tests per Capita by Estimated Undiagnosed HIV Infection per Capita, Ending the HIV Epidemic in the U.S. (EHE) Areas, 2019

936 FACTORS ASSOCIATED WITH HIV POSITIVITY UNAWARENESS AMONG TANZANIANS LIVING WITH HIV

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Background: The Tanzania HIV Impact Survey (THIS) 2016-2017 estimated that nearly four in every ten people living with HIV (PLHIV) aged 15 years and older were unaware of their HIV positive status. We assessed correlates of unawareness of HIV positive status among PLHIV in Tanzania.

Methods: We used data from the THIS 2016-2017, which was a cross-sectional nationally representative survey. In addition to self-reported HIV positive status, participants with detectable antiretrovirals (ARVs) in their blood were considered as aware. We used modified Poisson regression modeling to examine the associations of age, sex, residence, marital status, education, household wealth, condom use, and comprehensive HIV knowledge with unawareness of HIV-positive status.

Results: Among the 1,779 PLHIV in the sample, 39% were unaware of their HIV positive status after accounting for detectable ARVs in their blood. The risk of unawareness was 47% greater among males compared to females with adjusted prevalence ratio (aPR) of 1.47 [95% confidence interval (95%CI): 1.25-1.71]; 43% greater among young adults (15-24 years) compared to those who were at least 50 years old [aPR: 1.43; 95%CI: 1.05-1.93]; 42% greater among those who did not report condom use compared to those who did [aPR: 1.42; 95%CI: 1.19-1.71]; and 20% greater among those without comprehensive HIV knowledge compared to those with high knowledge [aPR: 1.20; 95%CI: 1.03-1.40]. The risk of unawareness was 26% lower among those who were widowed compared to those who were married or living together [aPR: 0.74; 95%CI: 0.56-0.98].

Conclusion: Our findings suggest that, in Tanzania, the risk of unawareness of HIV positive status was greater among PLHIV who were males, young adults, those who did not report condom use and those who had low HIV knowledge.
These findings confirmed preliminary descriptive survey results which led to a review of Tanzania’s identification strategies and reinforced targeted interventions to enhance HIV testing services by scaling up safe and ethical index testing, social network testing and HIV self-testing, focusing on at-risk populations including men and young adults.

937 OVER- AND UNDER-REPORTING IN HIV TESTING, STATUS, AND TREATMENT IN RURAL SOUTH AFRICA

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Background: Surveys are an important source of population-based indicators in tracking the HIV epidemic, including self-reported HIV status, testing history, and treatment adherence. Whether self-reported indicators are accurate has implications for epidemic monitoring but can be challenging to assess, particularly when collected without confirmatory biomarkers. We assess the accuracy of survey indicators by comparing self-reported testing, HIV status, and treatment responses in a population-based survey with clinical records in Bushbuckridge sub-district, South Africa.

Methods: We conducted a household-based survey of adults 18 – 49 years old in August – December 2018 with measures of HIV testing history, ART history, and source of health care. We drew from Agincourt Health and Demographic Surveillance System census records to link respondents to longitudinal clinical data from the 10 government facilities providing primary care in this area. We calculated indicators based on self-report and triangulated findings with data from clinical records. We adjusted testing estimates for known gaps in HIV test documentation and assessed individual and health system variables as predictors of reporting accuracy.

Results: Of 2089 survey participants, 1657 used a study facility and were eligible for analysis. Half of men and 84% of women reported an HIV test in the past year; 33% of reported tests could be confirmed in clinical data within 1 year and another 13% within 2 years. Confirmed tests were more common among women and younger adults. Accounting for documentation gaps, we estimated that 15% of men and 51% of women tested for HIV within the past year (Figure 1). Nearly half of those living with HIV did not report positive status; HIV prevalence was 16.2% based on self-report vs. 27.6% including clinic documentation. 238 of 250 (95.2%) of those self-reporting HIV diagnosis were verified against clinic documentation, ART history, and source of health care. We drew from Agincourt Health and Demographic Surveillance System census records to link respondents to longitudinal clinical data from the 10 government facilities providing primary care in this area. We calculated indicators based on self-report and triangulated findings with data from clinical records. We adjusted testing estimates for known gaps in HIV test documentation and assessed individual and health system variables as predictors of reporting accuracy.

Conclusion: Prevalence of recent HIV testing was substantially higher on self-report while reported prevalence of HIV was 10 percentage points lower than estimates including clinical records. Self-report overestimated current ART by at least 7 percentage points. Discrepancies were higher for men. While clinical records are imperfect, survey-based measures should be interpreted with abundant caution in this rural South African setting.

Figure: Prevalence of HIV testing within 12 months based on self-report and with clinic record confirmation

938 SIGNS OF LATE HIV DIAGNOSIS AND OUTBREAKS IN TRANSMISSION NETWORKS IN JAPAN

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Background: In Japan, approximately 30% of newly diagnosed HIV-infected individuals were identified after the onset of AIDS symptoms, suggesting that many people living with HIV (PLHIV) remain undiagnosed. Understanding factors that are associated with late HIV diagnosis (LHD), which is a major concern worldwide, is essential in achieving the 95-95-95 goal in Japan. Recently, we found that LHD in Japan was associated with a transmission clustering pattern. We present the possibility of identifying LHD-involving clusters in transmission-network analysis using our large-scale monitoring of domestic transmission clusters (dTCs) in Japan.

Methods: We monitored the dynamics of HIV-1 dTCs in Japan using cases collected by the Japanese HIV Drug Resistance Surveillance Network. dTCs dynamics and the network structure of newly diagnosed cases in Japan were monitored by our search program for HIV nationwide clusters by sequence (SPHNCS), which identifies the transmission link with the genetic distance estimated by the protease-reverse transcriptase sequences. We recruited 9,722 newly diagnosed cases between 2003 and 2021 from our surveillance network, and identified their dTC affiliation, network structure, and chronological tree. The relationship of the transmission network patterns to LHD and outbreak involvements, which were recognized by the CD4 count and chronological tree, were investigated.

Results: At the end of 2021, 566 subtype B and 105 CRF01_AE dTCs were registered in SPHNCS. Of these, seven and 30 clusters were recognized as LHD and outbreak involved clusters, respectively. At least 5,594 (57.5%) of newly diagnosed PLHIV in Japan were cases of LHD. Individuals in dTCs were more likely to be LHD cases than those not in dTCs (aOR, 0.80; p< 0.01). While mature transmission networks usually follow the power law with the lower degree being more frequent, outbreak clusters were characterized by the opposite degree distribution of the graph. In the degree distribution of the graph containing LHD, we observed a spike indicating a node at the center of the star-like structure in the network.

Conclusion: Our results indicated that the graph structure of HIV transmission networks may imply involvements in late HIV diagnosis and outbreaks. This suggests the possibility that monitoring dTCs dynamics using network analysis can quickly identify a local population where an outbreak occurred or testing was delayed, to promote preventive measures.

939 PATTERNS OF HIV-1 RECENT INFECTION AMONG GENERAL AND KEY POPULATIONS IN NIGERIA

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Background: HIV-1 Recent Infection Testing Algorithm (RITA) is increasingly applied to population-based surveys in numerous countries for population level estimates of HIV incidence. Nigeria commenced implementation of recency surveillance in 2020 and preliminary data for estimating state and national level HIV incidence is becoming available. The aim of the study is to understand the patterns of HIV-1 recent infection from inception of the implementation of HIV-1 recent infection surveillance in four states of Nigeria.

Methods: The study was a 30-month descriptive analysis of patterns of HIV-1 recent infection among newly diagnosed people living with HIV (PLHIV) who are 15 years old and above, who received RITA testing integrated into routine HIV Testing and Services (HTS) at activated sites. Data for Gombe, Kaduna, Kogi, and Lagos States were collected by the Japanese HIV Drug Resistance Surveillance Network. dTCs dynamics and the network structure of newly diagnosed cases in Japan were monitored by our search program for HIV nationwide clusters by sequence (SPHNCS), which identifies the transmission link with the genetic distance estimated by the protease-reverse transcriptase sequences. We recruited 9,722 newly diagnosed cases between 2003 and 2021 from our surveillance network, and identified their dTC affiliation, network structure, and chronological tree. The relationship of the transmission network patterns to LHD and outbreak involvements, which were recognized by the CD4 count and chronological tree, were investigated.

Results: At the end of 2021, 566 subtype B and 105 CRF01_AE dTCs were registered in SPHNCS. Of these, seven and 30 clusters were recognized as LHD and outbreak involved clusters, respectively. At least 5,594 (57.5%) of newly diagnosed PLHIV in Japan were cases of LHD. Individuals in dTCs were more likely to be LHD cases than those not in dTCs (aOR, 0.80; p< 0.01). While mature transmission networks usually follow the power law with the lower degree being more frequent, outbreak clusters were characterized by the opposite degree distribution of the graph. In the degree distribution of the graph containing LHD, we observed a spike indicating a node at the center of the star-like structure in the network.

Conclusion: Our results indicated that the graph structure of HIV transmission networks may imply involvements in late HIV diagnosis and outbreaks. This suggests the possibility that monitoring dTCs dynamics using network analysis can quickly identify a local population where an outbreak occurred or testing was delayed, to promote preventive measures.
between 20 and 44 years of age were recently infected with HIV compared with 38% of male within same age group. Similarly, 64% of males between ages 45 years and above were recently infected with HIV compared to 36% of their female counterpart. Recent infection rate was higher in the key population (3.8%; n=6,069) compared to the general population (2.7%, n=10,556). Disaggregating by sex, the rate of recent infection was higher among female (66%) in the general population compared to male (34%). However, the sex distribution of RITA recent was comparable among key population (51% in male versus 49% in female).

Conclusion: HIV-1 recent infection is higher in the key population, however, there is sex disparity in general population with female being more affected when compared to key populations. In general, recent infection rate may be higher among younger women and older men. The HIV response should prioritize preventive interventions targeting younger female at risk of HIV infection.

940 NO INCREASED VIOLENCE VICTIMIZATION AFTER RETURN OF RECENCY TEST RESULTS IN RWANDA

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Background: The U.S. President’s Emergency Plan for AIDS Relief does not recommend returning HIV recent infection results to newly diagnosed persons living with HIV (PLHIV) due in part to lack of safety evidence that return of results does not increase experience of intimate partner violence (IPV). We evaluated if IPV is more likely to increase as a result of return of recent infection results among newly diagnosed PLHIV in Rwanda.

Methods: We conducted a prospective cohort study of newly diagnosed PLHIV who underwent a rapid test for recent infection with baseline viral load to be collected at all sites and correlates of uptake of HIV testing were explored using logistic regression.

Results: Of 932 newly diagnosed PLHIV with IPV data from ≥1 visits after return of recent infection test results, 849 (91%) had LT infection and 83 (9%) had RT infection. Prevalence of IPV was higher at baseline before HIV diagnosis (29.8% vs. 17.6%, p< 0.001). Return of RT infection result was not associated with increased IPV compared to after HIV diagnosis (29.8% vs. 17.6%, p=0.4). Return of RT infection result was not associated with increased IPV compared to after HIV diagnosis (29.8% vs. 17.6%, p<0.001). Prevalence of IPV did not increase after return of HIV recent infection test result (17.6% vs. 16.1%, p=0.4).

Conclusion: IPV victimization did not increase after return of recent infection test result — RT or LT result — compared to return of HIV diagnosis in a context with high baseline IPV. Programs returning results can adopt strategies to mitigate IPV risks and ensure access to violence response services.

941 NON-HIV ENTRY POINTS TO DELIVER HIV SERVICES TO TRANSGENDER WOMEN IN INDIA

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Background: Transgender (TG) women in India have a 14 times higher burden of HIV compared to the general population. They also face substantial societal challenges with access to HIV prevention and treatment services. Further, for most transwomen access to hormone replacement therapy (HRT), cosmetics, and social entitlements take precedence over HIV services. Consequently, we established a comprehensive care model for the TG community including HIV and non-HIV services.

Methods: As part of a PEPFAR-funded program, three comprehensive community-led trans clinics (“Mitr Clinics”) were established in the Indian cities of Hyderabad, Pune and Thane since February 2021. These clinics provided HIV prevention and testing services with referral to the government program for free antiretroviral therapy (ART). The clinics also provided free consultation for HRT, cosmetics, and assistance with access to social protection schemes. Diagnostic for syphilis and nucleic acid testing for Chlamydia and N. gonorrhoeae were offered free of cost on-site. Client data was routinely collected at all sites and correlates of uptake of HIV testing were explored using logistic regression.

Results: Between February 2021–July 2022, 2276 individuals were registered across the 3 clinics. The majority (54%) had never received services as part of the government’s targeted interventions (TI) program. Median age was 26 years and 87% self-identified as a transwoman, 29% reported a history of transactional sex. The most utilized service was laser therapy (56%), followed by HIV services (54%). Of the total clients, 163 (7%) were aware of their HIV status at entry and 128 were currently on ART. 883 clients were screened for HIV at the clinics. HIV screening was significantly more common among those who visited the clinic for HRT (aOR 2.35; 95% CI 1.66, 3.33) or condoms (aOR 2.25; 95% CI 1.5, 3.37). 48 clients newly screened HIV positive at the clinic, of whom 20 completed confirmatory testing and 13 initiated ART (Figure). Additionally, 336 clients were tested for syphilis, 79 for CT and NG with a prevalence of 9%, 4%, and 0%, respectively.

Conclusion: These data highlight the role of integrating non-HIV services as entry points to generate demand for facilities from communities previously unreached by HIV programming. Integrating essential HIV and other STI services including confirmatory testing, ART and PrEP into such facilities will promote a “person-centric” approach to HIV care to this marginalized, vulnerable community.
HIV teams as a tool to improve HIV indicator condition-guided testing

By Carlijn Jordans, Diederik Janssen, Jeroen van Kampen, Femke Moilema, Jel Gisolf, Rachida El Moussouli, Gonneke Hermanides, Bart Rijnders, Annelies Verbon, Casper Roks

Figure - Clients' HIV Status and Testing Cascade

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**Background:** The majority of patients newly diagnosed with HIV in the Netherlands are late presenters and had multiple prior missed opportunities to test for HIV. To stop the HIV epidemic, adequate identification of people unaware of their HIV diagnosis is necessary. A proven key strategy, recommended by (inter)national guidelines, is indicator condition-guided testing. The aim of this study is to evaluate the impact of HIV teams on HIV indicator condition-guided testing in hospitals.

**Methods:** A single center prospective implementation project was conducted at Erasmus University Medical Center Rotterdam. A two-step approach was used to identify possible HIV indicator conditions by automatic ICD-10 screening, followed by cross-comparing with standardized health insurance (DB) codes. Data were collected on all patients ≥ 18 years who entered care between January 1st 2020 and June 12th 2022. Flagged indicator conditions were systematically reviewed by the HIV team. Multi-angle intervention started at August 1st 2020 and included proactive testing recommendations from the HIV team for patients treating patients with HIV indicator conditions. We evaluated HIV indicator condition prevalence and the impact of HIV teams on HIV testing rate overall, per interventional phase and per specialty.

**Results:** During the study period, a total of 218,271 diagnoses were newly registered. Of these, 18,743 (8.6%) were flagged as possible HIV indicator conditions. After manually reviewing, 2,026 HIV indicator conditions were identified. The overall HIV testing rate was 61.4% (1,244/2,026). In the pre-intervention period, the HIV testing rate was 43%, while after implementing HIV teams, the HIV testing rate was 52.1%, 80.7%, 68.7% and 70.4% for interventional phase 1, 2, 3, and 4, respectively. The overall HIV positivity rate was 0.7% (9/1,244) (pre-implement 0.4% and post-implementation 0.8%). Looking further at HIV test rates per specialty an increase in HIV testing rate was seen in all specialties with a peak in the first or second interventional phase (figure 1).

**Conclusion:** Implementing HIV teams increased the HIV testing rates with continued clinical benefit after an initial peak. Our data confirms a gap between indicator condition identification and HIV testing, even after proactive HIV testing advice. Future studies should focus on improving this gap and evaluate the barriers to test for HIV after HIV testing advice is given.

HIV testing rate per specialty - per period

- **0**% (9/1,244) (pre-implement 0.4% and post-implementation 0.8%).

**IMPACT OF MPXV OUTBREAK ON HIV TESTING IN A LARGE INTEGRATED HEALTHCARE SYSTEM

By Christopher Polk, Mindy Sampson, Jeremy Thomas, Robert Fairman, Tom Ludden, Hazel Tapp, Catherine Passaretti, Michael Leonard

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**Background:** People living with HIV (PLWH) were disproportionately impacted by the 2022 MPXV outbreak, with 40-60% of MPXV cases in PLWH. Due to this and substantial rates of sexually transmitted infection (STI) in patients diagnosed with MPXV we implemented a protocol for coinfection testing which included HIV testing at time of MPXV testing in 17 emergency departments (EDs) and 44 urgent cares (UC) across our large integrated healthcare system. As this HIV testing initiative overlaid an existing ED HIV testing program and EDs and UCs were the highest volume site for STI testing in our healthcare system, we examined the impact of the MPXV outbreak on HIV testing and diagnosis.

**Methods:** This is a retrospective observational study of HIV testing and diagnosis before and during the 2022 MPXV outbreak. We examined rates of HIV testing standardized by ED and UC visits during the Mpox outbreak from July to October 2022. We present data of the HIV teams and coinfection testing protocol as background.

**Results:** The majority of patients newly diagnosed with HIV in the Netherlands are late presenters and had multiple prior missed opportunities to test for HIV. To stop the HIV epidemic, adequate identification of people unaware of their HIV diagnosis is necessary. A proven key strategy, recommended by (inter)national guidelines, is indicator condition-guided testing.
1,202 through October 31,2022 compared to the prior 12 months: July 1, 2021 through June 30, 2022. We also compared total numbers of new HIV diagnosis during those time frames. T-tests were utilized to compare means of the tests and diagnoses before and during the outbreak. All analyses were conducted using SAS 9.4.

**Results:** The average number of HIV tests sent per month increased from 2.3 tests per 1000 patient visits per month prior to the Mpox outbreak to 3.8 tests per 1000 patient visits per month on average during the outbreak (p=.01). There was an average of 1.4 new cases of HIV diagnosed per month in our healthcare system’s EDs and UCs prior to the Mpox outbreak and an average of 3.9 new HIV diagnosis per month for the 4 months of the outbreak (p=.02). A higher number of tests per patient encounters occurred and more new HIV diagnosis were made in EDs compared to UCs both before and during the Mpox outbreak but testing and new diagnosis increased at both locations (see Figure). Of the 41 new cases of HIV diagnosed during the Mpox outbreak, 16 had testing sent concomitant with Mpox testing.

**Conclusion:** Significantly increased rates of HIV testing and new HIV diagnosis were observed during the Mpox outbreak in our healthcare system. This may have been the result of increased awareness of need for testing with increased provider education provided as part of the Mpox outbreak response. Further opportunities exist for strengthening confection testing for HIV and Mpox and identifying undiagnosed cases of both viruses.

**Figure:** Number of HIV tests per 1000 patient encounters and new HIV diagnosis by site and month

945 DNA-CONFIRMATION OF HIV-1&2 COINFECTIONS AMONG DUALLY-REACTIVE WEST AFRICAN PATIENTS

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The EDMARK-2 Study group

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**Background:** West Africa is characterized by the co-circulation of HIV-1 and HIV-2, leading to co-infections with both viruses. ART-naïve patients co-infected with both viruses were reported to experience a higher mortality rate compared to HIV-2 mono-infected patients. The accurate diagnosis of this co-infection remains challenging for the national HIV testing algorithms of West African countries, mainly due to limited access to DNA PCR technique. The aim of this study was to confirm HIV-1 and HIV-2 co-infection among patients serologically dually-reactive, DNA PCR testing.

**Methods:** A cross-sectional survey was conducted from April 2016 to October 2017 in the biggest HIV clinics of Côte d’Ivoire and Burkina Faso. A first serological confirmation was done in the referral laboratory using an in-house, indirect immuno-enzymatic assay allowing the qualitative detection of both HIV-1 and HIV-2 antibodies. In order to separately detect anti-HIV-1 and anti-HIV-2 antibodies, a type/group specific enzyme-immuno assay (HIV-GSEA) was used. To confirm the co-infections, HIV-1 and HIV-2 DNA-qualitative PCR assays were performed.

**Results:** A total of 91 patients were enrolled in the study and provided blood sample for HIV type confirmatory testing including 13 (14.3%) HIV-2 mono-reactive and 78 (85.7%) HIV-1/HIV-2 dually-reactive based on the HIV testing National Algorithms. The first serological ELISA confirmatory test performed showed that 80 (78.9%) of the 91 participants were dually-reactive. The HIV-GSEA performed on these 80 serum samples retrieve 61 HIV-1/HIV-2 dually-reactive samples. HIV-1 and HIV-2 DNA PCR were performed on 54 of the 61 HIV-1/HIV-2 dually-reactive samples and 46 out of 61 (75.4%) samples were found HIV-1/HIV-2 dually infected.

**Conclusion:** The contribution of type/group specific enzyme-immuno assay to accurately identify HIV-1/HIV-2 co-infections remain suboptimal, emphasizing the need for molecular diagnosis platforms in West Africa, to avail HIV DNA PCR test for the confirmation of HIV-1/HIV-2 co-infections.

**Study flow of characterization of HIV1&2 dually infected patients**

**946 EVALUATION OF A NOVEL MULTIPLEX IMMUNOASSAY: IMPROVEMENT FOR HTLV-2 DETECTION**

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**Background:** Despite the high sensitivity and specificity by confirmatory serological tests for HTLV infection, numerous cases with untyped or indeterminate results are reported, mainly for HTLV-2 infection. New approaches are urgently needed, especially among volunteer blood donors and individuals co-infected with some persistent viruses. Therefore, the aim of this study was to analyze a new Multiplex-ELISA (Multi-HTLV/InnYity Biomarkers) confirmatory serological methodology for HTLV-1.

**Methods:** For this purpose, we performed a comparative analysis between the molecular methodology of PCR-RFLP and Multiplex, with 254 plasma samples.

**Results:** In molecular assays (nested-PCR for HTLV-1/2), 128 samples were identified as positive for HTLV-1, 119 for HTLV-2 and 7 for co-infection of both types (HTLV-1+2). Among the samples confirmed as HTLV-1 by the gold standard methodology, 122/128 (95%) showed positive validation and reactivity to HTLV-1 discriminating proteins by the Multiplex; 4% (4/128) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results. For the co-infected samples (HTLV-1+2), the multiplex did not yield any results according to PCR-RFLP. Of these, 57.14% (4/14) had indeterminate or differently typed results to RFLP and only 0.78% (1/128) showed false-negative results. However, for the HTLV-2+ cases by molecular assays, 85% (101/119) were positive and correctly discriminated. Approximately 13% had indeterminate or incorrectly discriminated results and two cases (2/119; 1.7%) had false-negative results.
Clinical diagnosis is highly predictive of lab-confirmed mpox in a sexual health clinic

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Background: Sexual health clinics (SHC) played a central role in the diagnosis, prevention, and treatment of mpox throughout the 2022 epidemic. From June to October 2022, Public Health-Seattle & King County’s SHC diagnosed 44% of all mpox cases in King County, most of whom met tecovirimat treatment criteria. In July 2022, clinicians in our SHC began offering patients empiric tecovirimat therapy based on a clinical diagnosis of mpox or an intermediate clinical suspicion for mpox in a patient for whom arranging follow-up treatment would be challenging. We evaluated the accuracy of this approach and the extent to which it allowed us to rapidly initiate presumptive tecovirimat therapy.

Methods: Using data collected between July 29, 2022, and September 30, 2022, we estimated the sensitivity and positive predictive value (PPV) of the clinical diagnosis of mpox using mpox PCR positivity as a gold standard to define true infection. Additional analyses assessed the proportion of all mpox patients who received presumptive therapy and the proportion of presumptively treated patients who had a positive mpox test.

Results: Clinicians evaluated and sent lesion specimens for mpox testing for 320 patients, of whom 120 (38%) tested positive (Figure). Clinicians offered 101 (32%) of the 320 patients tecovirimat at their initial visit based on a clinical diagnosis. Clinical suspicion was high for mpox in 83 (82%) patients, 81 of whom elected to initiate empiric therapy. Clinical suspicion was only intermediate for mpox in 12 (12%) patients; all 12 patients were also offered tecovirimat, but none ultimately initiated. An additional 27 patients were offered tecovirimat after receipt of positive PCR results, of whom 26 (96%) initiated treatment. A total of 81 of the 101 patients with a clinical diagnosis of mpox tested PCR positive (PPV for clinical diagnosis = 80%), and clinical diagnosis was 79% sensitive (95 observed tecovirimat/120 total positives). All 12 patients with an intermediate clinical suspicion for mpox who were offered empiric tecovirimat tested PCR positive. Overall, 108 (90%) of all mpox patients initiated tecovirimat, 81 (75%) of whom started treatment on the day of their initial evaluation.

Conclusion: Clinical providers working in a high-volume, public SHC were able to accurately identify most patients with mpox to provide them with empiric tecovirimat, with 80% of presumptively treated patients ultimately testing positive for mpox.
Background: In the current outbreak, mpox is mostly spread through close or intimate contact, and gay, bisexual, and other men who have sex with men (MSM) are disproportionately affected. Integrating mpox testing with HIV/STI testing might be an opportunity to increase case finding. We describe HIV/STI and mpox testing among an online sample of sexually active MSM in the United States, including prevalence and access and barriers to testing.

Methods: We analyzed data collected during August 2022 from the American Men’s Internet Survey—Mpox Survey, a cross-sectional, online behavioral survey of 824 MSM in the United States, including prevalence and access and barriers to testing.

Results: Of 824 MSM, 126 (15.3%) reported at least one mpox-related symptom in the past 3 months; 88 (46.0%) with rash/sores, 57 (45.2%) with fever, and 11 (8.7%) with both. Increased prevalence of mpox symptoms was associated with condomless anal sex (CAS; aPR 1.53, 95% CI 1.06–2.20). Mpox testing was reported by 9/824 MSM (1.09%), including 5 with symptoms. Most MSM reporting mpox testing were non-Hispanic white (7/9 vs 1 Black, 1 Hispanic/Latino) and all 9 lived in urban areas. Most reported having an STI test (8/9), two or more partners (8/9), CAS (7/9), and group sex (6/9) in the last 3 months. Three were living with HIV; the remaining 6 not living with HIV reported current PrEP use. Of MSM with symptoms who didn’t report mpox testing but reported on mpox testing efficacy, 47/105 (44.8%) disagreed with the statement that they could get an mpox test if they wanted one. The most common barriers to testing were not knowing where to get tested (40/47, 85.1%) and difficulty getting appointments (23/47, 48.9%). Among those with high testing self-efficacy (58/105, 55.2%), the most common facilitators to testing were knowing where to test (52/58, 89.7%), convenient site hours (40/58, 69.0%), and low-cost testing (38/58, 65.5%).

Conclusion: Messages and interventions promoting testing awareness and community-based testing to increase access to convenient, low-cost services and improve mpox testing uptake are needed. An mpox neutral approach could integrate mpox testing and vaccination within PrEP and STI programs and incorporate targeted outreach to reduce barriers to mpox services for MSM in rural areas, Black and Hispanic/Latino MSM, and MSM living with HIV.

utility of a viral vesicular panel multiplex PCR assay for the diagnosis of mpox

Background: The ongoing Mpox outbreak is notable for its global reach and atypical presentations with significant variation in time of prodromal symptoms, staging and distribution of rash, and varied syndromic presentations (e.g., proctitis, pharyngitis). The overlap of the clinical presentation with common sexually transmitted infections and the high prevalence of co-infections (e.g., herpes simplex virus [HSV], varicella zoster virus [VZV]) highlights a need for improved diagnostic methods. A diagnostic test able to detect and differentiate Mpox from its common close mimics will be useful however, there are no multi-panel assays for vesicular and/or ulcerative lesions approved for clinical use. We evaluated a commercially available “research use only” multiplex PCR assay to detect Mpox virus, HSV, and VZV in clinical specimens.

Methods: Residual specimens collected during routine clinical care were tested using the multiplex panel (Qiagen, Germantown, MD, USA). This panel is a single-use automated multiplex real-time RT-PCR assay commercially available in the United States for “research use only” to detect Mpox virus Clade 1 (MPXV1), Mpox virus Clade 2 (MPXV2), HSV1, HSV2, HHV6, HEV, and VZV.
Reference testing was done by a commercial laboratory as part of routine care. Performance of the test assay was measured by calculating positive percent agreement and negative percent agreement with 95% confidence intervals using the efficient-score method (vassarstats.net).

Results: We tested 47 specimens from 40 unique patients (Table 1). The multiplex panel detected MPXV in 36/47 specimens whereas the reference standard detected MPXV in 37/47 (PPA 97.3%, 95% CI: 84.2-99.9%). The multiplex panel did not detect Mpxox in any specimen that was negative for the reference test (NPA 100%, 95% CI: 65.5-100%). Other viruses beyond Mpxox were detected by the multiplex panel in 11 samples, eight of which were co-infections with Mpxox according to the reference method.

Conclusion: We report the first validation in the clinical context of a commercially available multiplex PCR assay for the detection of Mpxox virus. The multiplex assay was highly accurate for the detection of Mpxox and offers several advantages over current assays; short-turn-around-time, ease of use, detection of other pathogens. Wider availability of Mpxox testing could shorten time to treatment and improve infection control interventions. Finally, multiplex assays that can detect Mpxox have utility in both disease surveillance and outbreak response.

Figure 1

<table>
<thead>
<tr>
<th>Reference Standard</th>
<th>Positive</th>
<th>Negative</th>
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</tr>
<tr>
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</tr>
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</tr>
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<td>Negative % Agreement</td>
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953 MPXO VIRAL LOAD BY SPECIMEN TYPE AND DIAGNOSTIC TESTING IN A CLINICAL LABORATORY

Nancy Matic, Matthew Young, Gordon Ritchie, Christopher F. Lowe, Marc G. Romney

Background: Unprecedented person-to-person transmission of MPXO (monkeypox virus) occurred in multiple countries in 2022, raising demand for diagnostic testing and generating uncertainty regarding optimal specimen types. A qualitative real-time PCR assay for the detection of MPXO DNA was validated and implemented in our clinical virology laboratory in August 2022, serving a high-risk population in both outpatient and inpatient settings.

Methods: The assay targeting the J2L/J2R gene (LightMix Modular Monkeypox Virus, TIB Molbiol), using MagnaPure96 for nucleic acid extraction and LightCycler 480 for amplification (Roche), was validated for swabs (skin lesions, rectal, genital, throat/oral) using reference specimens provided from a public health laboratory; all other specimen types were accepted but non-validated. All clinical specimens received for MPXO testing from Aug to mid-Dec 2022 were retrospectively analyzed for cycle threshold (Ct) values correlating with approximate MPXO viral load, percent positivity, and turnaround time.

Results: A total of 229 specimens were received from 144 unique patients (90.3% male, age range 4 to 83 years). Among patients testing positive for MPXO by any one specimen at first presentation (n=33), the mean Ct value and percent positivity varied by specimen type: Ct 22.3 for genital swabs (7/7, 100%), 22.4 for skin lesion swabs (16/16, 100%), 25.8 for rectal swabs (16/17, 94.1%), 30.3 for throat/oral swabs (12/13, 92.3%), 30.4 for urine (4/4, 100%), 35.0 for whole blood (6/7, 85.7%), and 36.3 for nasal/oropharyngeal swabs (1/1, 100%). 29.2% of patients (42/144) had more than one specimen type submitted at the time of initial presentation; of those with confirmed MPXO infection (n=17), only 3 patients (17.6%) had one of their specimens test negative for MPXO DNA (1 blood, 1 throat, and 1 rectal swab). The average turnaround time from specimen receipt to result was < 24 hours (20:12m). The overall positivity rate and testing volumes decreased from 14.7% (51/174 specimens) in Aug-Sep 2022 to 19.0% (8/42 specimens) in Nov-Dec 2022.

Conclusion: Swabs of skin lesions, particularly genital lesions, demonstrated the highest approximate MPXO viral load compared to other specimen types. Submitting multiple specimen types from patients did not improve diagnostic yield when skin lesions were otherwise present. Performing MPXO testing locally in a clinical laboratory enabled prompt turnaround times for timely diagnosis, clinical management and public health intervention.

954 MPXO VIRUS (MPXV) OUTBREAK IN BERLIN: IMPLEMENTATION OF MOLECULAR DIAGNOSTICS

Martin J. Obermeier, Ivanka Krznaric, Stefan Breuer, Kudor Harb, Robert Ehret

Background: In May 2022 an increasing number of MPXO infections has been reported throughout Europe. With increasing requests for MPXO diagnostics we implemented a multiplex PCR assay testing for Orthopox-Virus (OPV) and Monkeypox-Virus. For quality assessment an internal control and a cell control were included. The assay was validated against the national reference laboratory with more than 100 patient samples.

Methods: Outpatients with contact to confirmed cases or showing symptoms of MPXO infection were tested since end of May 2022 until mid of September. Samples consisted of swabs from lesions, genital, rectal and oropharyngeal swabs. To reduce the potential of false negative results we decided for a dual target approach including not only a PCR specific for MPXV but also including OPV. PCR primers and probe for OPV, MPXV and internal control (PHV-1) from TIB Molbiol (Berlin) were combined with primers and probes for 8-globin in one multiplex PCR. On a Biorad Cfx96 cycler using the TaqPath™ ProAmp™ Multiplex Master Mix run duration was 1:14 h.

Results: 2002 samples were tested for MPXV with 827 positive results. Turn around time was below 24 hours for more than 95% of the samples. The lower limit of detection was confirmed on an EQA panel to be below 60 cop/ml. Most of the positive results were detected in June and July with decreasing rates of tested samples in August and September (see figure). In some cases, with low concentration of MPXV DNA only one of the target genes could be detected (either MPXV or OPV). These cases were all confirmed by additional testing from a new swab. In 28 of the samples neither MPXV DNA nor cellular DNA could be detected.

Conclusion: We developed a rapid multiplex PCR system to improve patient care and allow better management of infection control. Despite information and vaccination campaigns since July 2022 we still detect new cases of MPXV infection including four cases of vaccination breakthroughs (single dose of vaccine) with high viral loads of up to 4 Mio. Copies/ml. High sensitivity of the assay is of great importance as quality of the swabs is divergent. MPXV molecular tests performed by week

955 PERFORMANCE OF A DUAL TARGET MPXO VIRUS REAL-TIME PCR ASSAY


Background: In May 2022 an increasing number of Monkeypox virus (Mpxo) cases in non-endemic countries, including the United States, was noted. This outbreak of Mpxo has primarily affected men who have sex with men who have reported recent sex with new or multiple partners. This study describes the performance of a single-well dual-target real-time PCR (RT-PCR) test for the detection of Mpxo without the need for tiered testing.

Methods: Lesion swabs collected in viral transport media (VTM) were extracted and tested by RT-PCR using a non-variola Orthopoxvirus target (E9L-NVAR) and an Mpxo virus clade II (West African) target (MPXV-WA). We assessed analytical sensitivity, specificity, precision, accuracy and specimen stability.
956 COVID-19 SELF-TESTING AMONG HEALTHCARE WORKERS AND GENERAL POPULATION IN MALAWI

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Background: COVID-19 testing is critical for identifying cases to prevent transmission. SARS-CoV-2 self-testing has the potential to increase diagnostic testing capacity and to expand access to hard-to-reach areas in low- and middle-income countries. We investigated the feasibility and acceptability of COVID-19 self-sampling and self-testing using SARS-CoV-2 Ag-RDT in Malawi.

Methods: Between July 2021 to February 2022, we conducted a mixed-methods cross-sectional study examining self-sampling and self-testing among a population of health care workers and adults. Participants were systematically sampled.

Results: Overall, 1,330 participants were enrolled of whom 674 (50.6%) were female with 664 for self-sampling and 666 for self-testing. Mean age overall was 30.7y (standard deviation [SD] 9.6). Self-sampling usability threshold for Standard Q was 273/333 (82.0%: 95% CI 77.4% to 86.0%) and 261/331 (78.8%: 95% CI 74.1% to 83.1%) for Panbio. Self-testing threshold was 276/335 (82.4%: 95% CI 77.9% to 86.3%) and 300/332 (90.4%: 95% CI 86.7% to 93.3%) for Standard Q and Panbio, respectively. Agreement between self-sample results and professional test results was 325/325 (100%) and 322/322 (100%) for Standard Q and Panbio, respectively. For self-testing, agreement was 322/323 (99.7%: 95% CI 98.3 to 100%) for Standard Q and 330/330 (100%: 95% CI 99.8 to 100%) for Panbio. Odds of achieving self-sampling threshold increased if the participant was recruited from a rural site (odds ratio [OR] 2.15 95% CI 1.44 to 3.23, P < .01). Compared to participants with primary school education those with secondary and those with tertiary achieved higher self-testing threshold OR 1.88 (95% CI 1.37 to 3.01), P = .01 and 4.05 (95% CI 1.20 to13.63), P = .02, respectively.

Conclusion: One of the first studies to demonstrate high feasibility of self-testing using SARS-CoV-2 Ag-RDTs in low- and middle-income countries potentially supporting large scale-up.

Table 1. Self-sampling and Self-testing Accuracy

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>%</th>
<th>SD</th>
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957 INTEGRATION OF SARS-CoV-2 RAPID ANTIGEN TEST IN HEALTH SERVICES IN KENYA AND CAMEROON

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Background: Early diagnosis of COVID-19 is key to prevent severe cases and poor outcomes in vulnerable populations, including pregnant women and people living with HIV or infected with tuberculosis (TB). The feasibility of integration of SARS-CoV-2 antigen rapid diagnostic testing (Ag-RDT) into maternal neonatal, and child Health (MNH); HIV; and TB clinics is unknown.

Methods: We analyzed data from a SARS-CoV-2 screen and test program implemented in 50 health facilities (25 in Kenya and 25 in Cameroon), integrating SARS-CoV-2 Ag-RDT in MNH, HIV, and TB clinics between May and October 2022. Clients aged two and older attending MNH, HIV, and TB clinics were offered SARS-CoV-2 screening, and those eligible were tested using SARS-CoV-2 Ag-RDT. Routine SARS-CoV-2 program data were captured through dedicated paper forms in Cameroon or an electronic medical record (EMR) interface in Kenya and transferred to a database for analysis. We estimated the proportion of clients screened and tested and the SARS-CoV-2 positivity rates.

Results: Overall, 527,184 attendee visits were reported in Cameroon (282,404) and Kenya (244,780), with screening for COVID-19 symptoms and exposure performed in 256,033 (48.5%) with substantive variations between countries (62.6% in Cameroon and 32.4% in Kenya). Among the 256,033 screened, 19,058 (7.4%) were eligible for testing (9.0% in Cameroon and 3.9% in Kenya), of whom 12,925 (67.8%) were tested for SARS-CoV-2 with substantial variation in testing rates between countries (61.9% in Cameroon and 97.9% in Kenya) and clinics were offered SARS-CoV-2 screening, and those eligible were tested using SARS-CoV-2 Ag-RDT. Routine SARS-CoV-2 program data were captured through dedicated paper forms in Cameroon or an electronic medical record (EMR) interface in Kenya and transferred to a database for analysis. We estimated the proportion of clients screened and tested and the SARS-CoV-2 positivity rates.

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Factors associated with COVID-19 screening

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>1.20 (0.80 - 1.84)</td>
<td>1.10 (0.68 - 1.79)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Georgia</td>
<td>1.60 (1.09 - 2.34)</td>
<td>1.50 (0.98 - 2.30)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Zambia</td>
<td>1.80 (1.16 - 2.78)</td>
<td>1.70 (1.06 - 2.71)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

958 IMPACT AND COST-EFFECTIVENESS OF COVID-19 RAPID SELF-TESTING STRATEGIES IN SCHOOLS

Joshua M. Chevalier1, Alvin X. Han1, Megan A. Hansen1, Ethan Klock2, Hiromi Panditikhorale1, Tom Ockhuisen2, Sarah Girdwood3, Nkogemeleng Lekodeba4, Alexandra de Nooy3, Helen E. Jenkins1, Colin A. Russell5, Brooke Nichols1

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Background: Despite widespread vaccination and increasing population immunity from previous infections, community transmission of COVID-19 continues, and testing may continue to be an important component of our response particularly with the proliferation of new variants of concern. Strategic deployment of SARS-CoV-2 antigen-detection rapid diagnostic (AgRDT) self-tests to settings with increased transmission potential can reduce the viral burden within the specific settings, such as in K-12 schools, and may have spillover benefits for broader community transmission.

Methods: Using a previously developed agent-based simulation model, parameterized to three distinct country archetypes (Brazil, Georgia, Zambia), we analyzed 11 different self-testing strategies within the school-going population at three testing frequencies under 24 different epidemic conditions (Rt, vaccination coverage/effectiveness), comprising a total of 696 scenarios per country. Strategies included symptomatic testing, and in addition, asymptomatic testing at 5, 20, 40 or 100% of schools, or asymptomatic contact testing. These were all targeted to either only teachers or teachers and students.

Then, with the cost to offer a COVID-19 self-test in schools at USD 2.50, we performed an economic analysis with all scenarios to identify the most cost-effective strategies by country.

Results: Routine asymptomatic testing of teachers and students at 100% of schools reduced the greatest number of infections across contexts, but at the highest cost. However, with respect to both the reduction in infections and total cost, symptomatic testing of all teachers and students appears to be the most efficient strategy. Symptomatic testing can prevent up to 69.3%, 64.5%, and 75.5% of school infections in Brazil, Georgia, and Zambia, across all epidemic conditions. Additionally, it can prevent up to 77,200, 80,900, 107,800 asymptomatic days per 100,000 teachers and students in each country, respectively, over the course of a 90-day epidemic wave. The incremental cost-effectiveness ratios for strategies that consistently appeared on the cost-effectiveness frontier across countries and epidemic conditions are shown in Figure 1 for an Rt of 1.2.

Conclusion: If financial resources are limited, symptomatic testing of teachers and students has the potential to be cost-effective while reducing a substantial number of infections and the amount of time lost from the classroom, making it a feasible strategy for implementation in a variety of settings.

Incremental cost-effectiveness ratios of COVID-19 self-testing strategies in schools by country at Rt 1.2 and vaccination effectiveness 30%

959 COST-EFFECTIVENESS OF WORKPLACE COVID-19 SELF-TESTING: A MATHEMATICAL MODELING STUDY

Ethan Klock1, Alvin X. Han1, Joshua M. Chevalier1, Megan A. Hansen1, Hiromi Panditikhorale1, Tom Ockhuisen2, Sarah Girdwood3, Nkogemeleng Lekodeba4, Alexandra de Nooy3, Helen E. Jenkins1, Colin A. Russell5, Brooke Nichols1

1Boston University, Boston, MA, USA, 2Amsterdam University Medical Center, Amsterdam, Netherlands, 3FIND, Cape Town, South Africa, 4Health Economics and Epidemiology Research Office, Johannesburg, South Africa, 5Foundation for Innovative New Diagnostics, Geneva, Switzerland

Background: As COVID-19 cases persist, one potential intervention to reduce absenteeism in the workplace due to COVID-19 is to use rapid antigen diagnostics to mitigate the spread of SARS-CoV-2. Furthermore, routine testing in the workplace offers an avenue to reaching a large proportion of the population which could lead to a greater community impact beyond solely mitigating transmission events that occur in the workplace. We sought to identify the most cost-effective workplace testing strategies at the community level and within individual workplaces.

Methods: We used two models to understand how SARS-CoV-2 AgRDTs could best be implemented within the workplace to mitigate the spread of COVID-19. In our community-level dynamic transmission model, PATAT, we evaluated the impact of symptomatic testing and asymptomatic testing of a fixed proportion of the formally employed workforce on broader community transmission. We stratified runs by asymptomatic testing frequency, vaccine coverage, vaccine effectiveness, and Rt. Simulations were informed using demographic data from Georgia, Brazil, and the Netherlands. We conducted a cost-effectiveness analysis using the results from each country and assumed a $2.50 total cost per test.

Results: We observed a substantial decrease in the number of infections occurring in both the workplace and community when a SARS-CoV-2 AgRDTs strategy was implemented. Under all conditions, mandatory symptomatic testing and related quarantine from the workplace averted up to 72%, 79%, and 74% of community infections in Brazil, Georgia, and the Netherlands respectively. Limiting tests to symptomatic workers was always on the cost-effectiveness frontier, regardless of the vaccine coverage, efficacy, or Rt of the virus (Figure 1), at $2-$4 per workplace infection prevented. While asymptomatic testing was on the cost-effectiveness frontier, it would cost an additional $500-$6700 to prevent one additional workplace infection. The added benefit of routine asymptomatic testing was minimal until 100% of the workforce was reached.

Conclusion: We found self-testing with AgRDTs for the formally employed workforce is both efficient at reducing workplace and community infections as well as cost-effective when targeting symptomatic individuals. Willingness to pay to avoid workplace absenteeism may differ by country, individual workplaces, and the perceived economic value of several workdays missed. If there is a higher willingness to pay, routine asymptomatic screening may be considered.
960 HOME VERSUS FACILITY SARS-CoV-2 TESTING AMONG OLDER ADULTS IN NEW YORK CITY

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1ICAP at Columbia University, Brooklyn, NY, USA, 2ICAP at Columbia University, New York, NY, USA, 3ICAP at Columbia University, Johannesburg, South Africa, 4ICAP at Columbia University, New York, NY, USA

Background: While nationwide SARS-CoV-2 testing in the United States shifted from facility- to home-based in 2021, less is known about testing behavior of older adults who live at home. We analyze characteristics of older adults who reported facility-based testing with those who tested only at home or at both locations.

Methods: Adults 70 years and older living at a home with a landline in New York City were selected using random digit dialing and completed a COVID-19 survey from February 2022 – March 2022. We conducted descriptive statistics using survey weights and bi-variable and multivariable analyses.

Results: Overall, 237 of a total of 294 (81%) participants had tested for SARS-CoV-2 in the prior year. Among those who tested, 81% had tested only at a facility, 4% only tested at home, and 15% tested at both locations (home and facility). White participants more often reported testing at both locations (27%) compared to Black (7%), Latinx (7%) or participants of another race (11%; p-value: 0.004). Those with college education or higher were less likely to rely solely on facilities for testing (75%) compared to those with less education (91%; p-value: 0.02) and 38% of those who reported currently working had tested both locations compared to only 12% of those who were not working (p-value: 0.002). There were no differences in testing by age, sexual orientation, or self-reported mobility. A multivariable logistic regression model that compared those who only tested at a facility with those who tested at both locations or only at home found that when adjusting for working status, age group and education, compared to White participants, Black participants had a third (0.33) the odds of white participants of testing at home or at both locations (p-value: 0.026).

Conclusion: In this sample of urban older adults, using a facility for SARS-CoV-2 testing was more frequently reported than testing at home, indicating the need to retain facility-based testing for this population. However, White participants, more educated participants and those who were working more frequently reported home-based testing compared to other groups suggesting that social constraints may limit access to home testing among the latter group of older adults.

Socio-demographic profile by testing location among older New York City adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Home only</th>
<th>Facility only</th>
<th>Both</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>77.7</td>
<td>80.3</td>
<td>80.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Race and ethnicity</td>
<td>White</td>
<td>Black</td>
<td>Latinx</td>
<td>0.002</td>
</tr>
<tr>
<td>Race</td>
<td>82.1</td>
<td>73.7</td>
<td>67.7</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td>55–64</td>
<td>65–74</td>
<td>75+</td>
<td></td>
</tr>
<tr>
<td>White ethnicity</td>
<td>90.3</td>
<td>89.0</td>
<td>89.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Education</td>
<td>Less educated</td>
<td>College graduate</td>
<td>79.7</td>
<td>81.9</td>
</tr>
<tr>
<td>Employment status</td>
<td>Working</td>
<td>Not working</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently working</td>
<td>58.4</td>
<td>54.1</td>
<td>44.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Not currently working</td>
<td>41.6</td>
<td>45.9</td>
<td>55.9</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>Limited</td>
<td>Very active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited</td>
<td>82.5</td>
<td>69.6</td>
<td>75.4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Cost-Effectiveness of Workplace Testing Strategies at $R_t = 1.2$ With Low Vaccine Efficacy by Workplace Infections Averted

961 ANTIBODIES TO SARS-CoV-2 VARY DUE TO ORDER AND FREQUENCY OF VACCINATION OR INFECTION

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

Background: Sero-studies of SARS-CoV-2 have used antibody (Ab) responses to spike (S) and nucleocapsid (N) antigens to differentiate mRNA vaccinated (S+ or N+) from infected (S+ / N-) individuals. We performed testing on well-characterized subjects to determine how repeated vaccination or infection, and time from those exposures, influence these Ab levels.

Methods: Samples from individuals with known infection status: pre-pandemic negative controls n=462; first-time infected n=237 (~45 days post); vaccinated after infection n=34 (~40 days post-vaccination and ~180 days post-infection); fully vaccinated n=158 (~50 days post); boosted n=31 (~30 days post); breakthrough n=18 (~14 days post-infection); reinfected n=10 (varied). Longitudinal samples (n=51) from subjects with evidence of reinfection (symptoms and/or positive rapid antigen test), were tested to determine the impact of the order of infection and/or vaccination on the magnitude of the anti-S and anti-N IgG Ab detected in the blood. Testing was performed with Mesoscale Diagnostics (Gaithersburg, MD) assay. Outcomes are presented in WHO International Binding Antibody Units (BAU/mL). The cutoff for a positive result was 18 BAU for S and 12 BAU for N.

Results: The median amount of Ab (IQR) in BAU for each group (Figure A) was: pre-pandemic negative controls S 0.530(0.271,0.13), N 0.550(0.181,1.67); first-time infected S 114(51,328), N 70(29,229); vaccinated after infection S 4367(2479,4837), N 157(73,353); fully vaccinated S 998(586,1529), N 31016.0,68; boosted S 2988(1768,3522), N 0.590.32,1.03; breakthrough S 2429(2032,3413), N 2.5(0.93,8.6); reinfected S 1533(486,4643), N 7.8(2.6,62). For the breakthrough and second infections 17% and 40% were seropositive to N, respectively. Longitudinal analysis (Figure B) of those with multiple infections showed that all those with a positive rapid antigen test for their second infection had an increase in N Ab.

Conclusion: The prevalence of antibodies to nucleocapsid cannot be used to determine the proportion of individuals infected to SARS-CoV-2 in a vaccinated population. Booster, repeated, and breakthrough infections are associated with IgG Ab levels to S > 400 BAU/mL. A majority of breakthrough infections did not elicit an Ab response to N. For those with repeated infection, a minority elicited antibody responses to N. This could be related to misdiagnosis or the burden of infection, as only those who were positive by rapid antigen assay (indicative of a high viral load) had an increase in N Ab.

Figure: Longitudinal levels of antibody against SARS-CoV-2 spike and nucleocapsid antigen in reinfected individuals.
SARS-CoV-2 REPLICON SYSTEM FOR THE PHENOTYPIC EVALUATION OF GENE SUBSTITUTIONS

**Methods:** The SARS-CoV-2 replicon protocol was adapted and optimized based on (Zhang 2021). The replicon RNA was produced by in vitro transcription of full-length replicon DNA assembly by ligation of plasmid fragments encoding for the SARS-CoV-2 non-structural proteins (Nsp), nucleoprotein and gaussia luciferase reporter protein. Wild-type and mutant replicon RNAs were transfected into HuH7-1CN cells by electroporation and treated with remdesivir (RDV). To determine EC50 values, luciferase activity was determined at 48 hours post transfection. A recombinant SARS-CoV-2 virus rescue system (Xie 2020) was used to generate matching Nsp mutants for comparison with the replicon system.

**Results:** The selected substitutions reflective of Omicron BA.5 sub-lineage BF7 variant: the triple mutants (Nsp12 (P323L) + Nsp13 (R392C) + Nsp14 (I42V), and a single Nsp12 L247F mutant as well as several specific Nsp12 mutations identified in vitro resistance selection with RDV or RDV parent nucleoside analog GS-441524 were cloned into the replicon and tested for susceptibility to RDV. RDV inhibited the SARS-CoV-2 wild-type replicon with a mean EC50 value of 14.7 ± 3.5 nM (N=9). The Nsp12 P232L substitution, a common polymorphism in all major variants of concern including Omicron, was fully susceptible to RDV with a 0.6-fold change in EC50 from the wild-type. The Omicron BF7 triple mutants and L247F were also fully susceptible to RDV with 0.5- and 0.4-fold changes, respectively. Nsp12 substitutions F480L, V557L, V792L, S759A+V792L, and C799F resulting from in vitro resistance selections with RDV showed minimal to moderate levels of reduced susceptibility to RDV (1.8 to 18.3-fold change) (Table 1). The RDV EC50 fold changes correlated between the non-infectious replicon and recombinant infection virus system (Table 1).

**Conclusion:** The replicon system is a convenient and reproducible model to test the susceptibility of SARS-CoV-2 mutant variants to RDV and potentially other antivirals. The common Nsp12 polymorphisms in all variants including the highly transmissible Omicron variant were fully susceptible to RDV. Table 1. Correlation of EC50 fold changes between the SARS-CoV-2 replicon and recombinant virus system

<table>
<thead>
<tr>
<th>Nsp12</th>
<th>RDV EC50 (nM)</th>
<th>Average fold change (N=7)</th>
<th>Fold change relative to WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>17.0 ± 11.0</td>
<td>16.9 ± 9.1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>P232L</td>
<td>44.0 ± 2.8</td>
<td>8.4 ± 2.0 (0.8)</td>
<td></td>
</tr>
<tr>
<td>F480L</td>
<td>118.4 ± 26.2</td>
<td>28.9 ± 4.8 (0.5)</td>
<td></td>
</tr>
<tr>
<td>V557L</td>
<td>112.4 ± 27.0</td>
<td>28.9 ± 4.8 (0.5)</td>
<td></td>
</tr>
<tr>
<td>V792L</td>
<td>173.1 ± 70.7</td>
<td>13.7 ± 4.8 (0.8)</td>
<td></td>
</tr>
<tr>
<td>S759A/V792L</td>
<td>105.9 ± 13.1 (1.1)</td>
<td>108.5 ± 10.5 (1.0)</td>
<td></td>
</tr>
<tr>
<td>C799F</td>
<td>198.4 ± 81.1</td>
<td>20.0 ± 2.4 (0.5)</td>
<td></td>
</tr>
</tbody>
</table>

**Methods:** We used an agent-based community assessment model, Propelling Action for Testing and Treatment, to estimate how various strategies of asymptomatic Ag-RDT self-testing of a fixed percentage of persons attending large religious gatherings (10%, 20%, 40%, 100%), in addition to the general underlying level of ongoing symptomatic testing in the population, would impact community transmission of SARS-CoV-2 in 3 contexts (Brazil, Georgia, Zambia). These testing strategies were analyzed with bi-weekly and weekly asymptomatic self-testing in a population with varying levels of vaccine efficacy (low/high), vaccine coverage (10%, 50%, 80%), and reproductive numbers (0.5, 1.2, 1.5, and 2.0) to simulate varying stages of the COVID-19 pandemic. We then performed an economical evaluation of the results from the model to understand the impact and cost-effectiveness of each self-testing strategy at places of worship.

**Results:** In each of the epidemic conditions modeled, testing of symptomatic persons at weekly and biweekly frequencies can avert 2%-16% of Brazilian community infections and 31%-45% of infections occurring in places of worship in Brazil. The same is true in Georgia (1%-6% of total infections and 28%-45% place of worship-related infections) and Zambia (2%-21% of total infections and 25%-45% of place of worship related infections) despite differences in the proportion of populations regularly attending places of worship in the 3 countries. Asymptomatic self-testing in 100% of places of worship in a country result in the greatest percent of infections averted and consistently lands on the cost-effectiveness frontier yet requires a budget $250-1550x greater than that of symptomatic testing alone.

**Conclusion:** Testing of symptomatic persons attending regular religious gatherings have a significant impact on the spread of SARS-CoV-2 in places of worship and can significantly reduce community spread in contexts where population level attendance at religious gatherings is high. Cost-effectiveness analysis from Brazil, Georgia and Zambia modelling results with infections averted within places of worship and total community infections averted assuming a total cost per self-test of $2.50 USD.

**Table 1:** Correlation of EC50 fold changes between the SARS-CoV-2 replication and recombinant virus system

**GATHERINGS IN LICs & MICS**

**Methods:** In 49 high attendance facilities in Kiambu County identified as possible points of community-based transmission, individuals two years old and older were offered COVID-19 testing and vaccination. Those accepting testing were enrolled in the study after providing written informed consent. A questionnaire was administered and a nasopharyngeal swab was collected. Those testing positive and those testing negative but with COVID-19 symptoms were referred for PCR testing and genome sequencing. Data were analyzed using descriptive statistics. The total cost of implementing the community
testing was estimated from a health system perspective using a micro-costing method.

**Results:** From June-September 2022, 4,062 individuals were offered testing (mean age 39 years, 2,114 (58.6%) were male). The testing acceptance was 78.1% (3,174/4,062) 95%CI); 76.9%–79.5%). The case detection rate was 34/3,174 (1.07%; 95%CI 0.7%–1.4%). Table 1 shows the testing and case detection rates by facility type. Of the 34 positive cases, 11 (32%) were asymptomatic. A PCR result was available for 27 Ag-RDT positive participants and 13 Ag-RDT negative participants with SARS-CoV-2 symptoms and was positive in 24 (88.9%) and 4 (30.8%) respectively. Circulating variants were identified in 11 participants (Omicron 22A: 36% and 22B: 64%); 15 samples could not be sequenced due to CT values >35. Community mobilization was the major cost driver (26%) followed by the purchase of SARS-CoV-2 Ag-RDT (20.5%). The total cost of the intervention was US$50,538; the cost per individual tested was US$15.89 and US$1,484 per new COVID-19.

**Conclusion:** Targeted mass community testing using SARS-CoV-2 Ag-RDT is a feasible and affordable strategy in identifying priority areas for vaccination and early treatment for individuals with COVID-19.

Table 1. Testing and case detection rates in the different venue types

<table>
<thead>
<tr>
<th>Venue type</th>
<th>Number tested</th>
<th>Percentage testing</th>
<th>Number tested with positive result</th>
<th>Percentage positive amongst tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markets/shopping centers</td>
<td>2595 (65.6)</td>
<td>1906 (73.4)</td>
<td>24 (1.2)</td>
<td>1.3%</td>
</tr>
<tr>
<td>Child's camp</td>
<td>630 (67.7)</td>
<td>462 (73.9)</td>
<td>2 (0.4%)</td>
<td>0.08%</td>
</tr>
<tr>
<td>Bus stops</td>
<td>397 (67.2)</td>
<td>295 (74.0)</td>
<td>0 (0.0%)</td>
<td>0.02%</td>
</tr>
<tr>
<td>Stadiums</td>
<td>656 (76.4)</td>
<td>509 (77.4)</td>
<td>0 (0.0%)</td>
<td>0.00%</td>
</tr>
<tr>
<td>Others (n=207)</td>
<td>598 (79.5)</td>
<td>512 (85.1)</td>
<td>0 (0.0%)</td>
<td>0.00%</td>
</tr>
<tr>
<td>Overall (n=2348)</td>
<td>4661 (58.2)</td>
<td>3214 (69.2)</td>
<td>34 (0.7)</td>
<td>0.77%</td>
</tr>
</tbody>
</table>

965 SARS-CoV-2 WASTEWATER SURVEILLANCE IN A US JAIL: CORRELATION, FEASIBILITY, NEXT STEPS

**Lindsay Saber,** Anne Spaulding

SWANSS Study Group

**Emory University, Atlanta, GA, USA**

**Background:** Jails house vulnerable persons. Crowded conditions, restricted access to medical care, and limited resources facilitate infectious disease outbreaks, particularly for airborne, highly transmissible diseases like COVID-19 (C19). Wastewater-Based Surveillance (WBS) is a low-cost, highly sensitive, non-invasive method that can provide an early warning of C19 surges in communities. We examined the value of SARS-CoV-2 WBS for a mega-jail.

**Methods:** 28-week study period: 10/20/21-5/5/22. Wastewater samples were collected x 25 weeks; SARS-CoV-2 RNA was measured using RT-qPCR. We sampled one manhole serving multiple housing units. C19 rapid test data on jail residents was used to assess the predictability between paired Ct values and weekly % of positive samples. Weekly WBS results for C19 correlated with the proportion of C19 diagnosed cases (aOR, 2.87; 95% CI, 2.16-3.80; p<0.0001). The Spearman correlation coefficient between weekly SARS-CoV-2 RNA in wastewater and % of positive samples was r= 0.628; linear regression likewise showed a similar correlation.

**Conclusion:** Weekly WBS results for C19 correlated with the proportion of C19 individual test results. WBS proved to be a practical strategy to surveil for C19 in this jail setting. We are developing methods to identify exact source, by housing unit, of wastewater with positive signal. Future studies will explore WBS for Mpxv and HIV in correctional facilities. HIV RNA can be found in wastewater specimens; whether WBS for HIV in congregate facilities is feasible remains an open question.

966 DISABILITY AND COVID-19 TESTING: A CROSS-SECTIONAL RADx-UP STUDY

**Haley R. Martin,** Wensong Wu1, Sabrina Sales Martinez2, Jose A. Bastida Rodriguez2, Angelique Johnson3, Marianna K. Baum4

1Honda International University, Miami, FL, USA, 2Honda International University, Miami Beach, FL, USA

**Background:** Nearly 26% of adults in the U.S. live with disability and are more likely to experience chronic health conditions, barriers to healthcare, and severe COVID-19 illness. Therefore, COVID-19 testing of adults living with disability is important to consider. The purpose of this study was to explore relationships between disability and COVID-19 testing, infection, and related challenges.

**Methods:** A Rapid Acceleration of Diagnostics—Underserved Population (RADx-UP) project in Miami, FL determined disability with a modified version of the Washington Group General Disability Measure. HIV serostatus and COVID-19 vaccination were confirmed with medical records. COVID-19 testing and infection history were self-reported. Statistical analyses included chi-squared tests and multiple binary logistic regression; variance inflation factors were calculated to ensure absence of collinearity.

**Results:** A total of 1,689 RADx-UP participants with an average age of 55±12.3, 3,5% male, 49% Black non-Hispanic, 23% living with HIV (86% virally suppressed), and 76% received at least one dose of a COVID-19 vaccine. Nearly 40% were disabled, 37% reported employment disability, and 21% were functionally disabled (disability that interferes with performance of daily activities). Despite recruitment from the same sources, PLWH, compared to those without HIV, were more likely to be disabled (52% vs 36%; p<0.0001), report employment disability (63% vs 30%; p<0.0001), and report functional disability (29% vs 18%; p=0.0001). Those with employment disability were less likely to have ever been tested for COVID-19 compared to those without (81% vs 85%; p=0.026). Employment disability was also associated with lower odds of having ever tested positive for COVID-19 after adjustment for demographics, health insurance, HIV, COVID-19 vaccination, smoking, and lung disease (aOR, 0.62; 95 CI, 0.43-0.90; p=0.013). Disability was associated with greater odds of transportation challenges (aOR, 2.33; 95 CI, 1.76-3.08; p<0.001), illicit drug use (aOR, 1.92; 95 CI, 1.49-2.47; p<0.0001), and smoking (aOR, 1.74; 95 CI, 1.39-2.17; p<0.0001). Compared to those without, those with transportation challenges (14% vs 40%; p<0.0001) and illicit drug use (18% vs 30%; p=0.001) were more likely to postpone medical care.

**Conclusion:** Lower COVID-19 testing rates may contribute to underestimated COVID-19 positivity rates in adults living with disability. Challenges with transportation and substance abuse contribute to less engagement in care.

967 COST-EFFECTIVENESS OF THE DUAL PREVENTION PILL FOR CONTRACEPTION AND HIV PROPHYLAXIS

**Masabho P. Mialili,** David Kaftan1, Ingrida Platai1, Hae-Young Kim1, Danielle Resar1, Danny Edwards2, Jenny Campbell3, Sarah Jenkins4, Anna Bershteyn5

1Department of Population Health, NYU Grossman School of Medicine

**Background:** Nearly 50% of women in the U.S. live with disability and are more likely to experience chronic health conditions, barriers to healthcare, and severe COVID-19 illness. Therefore, COVID-19 testing of adults living with disability is important to consider. The purpose of this study was to explore relationships between disability and COVID-19 testing, infection, and related challenges.

**Methods:** A Rapid Acceleration of Diagnostics—Underserved Population (RADx-UP) project in Miami, FL determined disability with a modified version of the Washington Group General Disability Measure. HIV serostatus and COVID-19 vaccination were confirmed with medical records. COVID-19 testing and infection history were self-reported. Statistical analyses included chi-squared tests and multiple binary logistic regression; variance inflation factors were calculated to ensure absence of collinearity.

**Results:** A total of 1,689 RADx-UP participants with an average age of 55±12.3, 3,5% male, 49% Black non-Hispanic, 23% living with HIV (86% virally suppressed), and 76% received at least one dose of a COVID-19 vaccine. Nearly 40% were disabled, 37% reported employment disability, and 21% were functionally disabled (disability that interferes with performance of daily activities). Despite recruitment from the same sources, PLWH, compared to those without HIV, were more likely to be disabled (52% vs 36%; p<0.0001), report employment disability (63% vs 30%; p<0.0001), and report functional disability (29% vs 18%; p=0.0001). Those with employment disability were less likely to have ever been tested for COVID-19 compared to those without (81% vs 85%; p=0.026). Employment disability was also associated with lower odds of having ever tested positive for COVID-19 after adjustment for demographics, health insurance, HIV, COVID-19 vaccination, smoking, and lung disease (aOR, 0.62; 95 CI, 0.43-0.90; p=0.013). Disability was associated with greater odds of transportation challenges (aOR, 2.33; 95 CI, 1.76-3.08; p<0.001), illicit drug use (aOR, 1.92; 95 CI, 1.49-2.47; p<0.0001), and smoking (aOR, 1.74; 95 CI, 1.39-2.17; p<0.0001). Compared to those without, those with transportation challenges (14% vs 40%; p<0.0001) and illicit drug use (18% vs 30%; p=0.001) were more likely to postpone medical care.

**Conclusion:** Lower COVID-19 testing rates may contribute to underestimated COVID-19 positivity rates in adults living with disability. Challenges with transportation and substance abuse contribute to less engagement in care.
Background: Women in sub-Saharan Africa (SSA) experience the world’s highest rates of both HIV infection and unintended pregnancy. The Dual Prevention Pill (DPP) co-formulates PrEP and oral contraception into a single daily pill and may be preferred by women with dual prevention needs. However, most countries in SSA face severe healthcare resource constraints. Research is needed to assess whether, in what populations, and in what use cases the DPP would be cost-effective.

Methods: We augmented an agent-based SSA HIV model with maternal health parameters including unintended pregnancy, abortion, and maternal mortality. Based on a previous market analysis, we assumed a primary DPP user population of current oral contraceptive users ages 25–49, and alternative user populations with different risk profiles (ages 15–24, sex workers, serodiscordant couples) and product use profiles (unmet need for contraception, oral PrEP use, and condom use). For each population and use case, we estimated the HIV infections averted, pregnancies averted, disability-adjusted life-years (DALYs) averted, and incremental cost-effectiveness ratio (ICER) over a 30-year time horizon, 3% annual discount rate, and assuming equivalent adherence to DPP as to oral contraceptives. Sensitivity analyses explored different adhered levels, unit costs, time horizons, economic discount rates, and SSA settings.

Results: The DPP is likely to be cost-effective in current oral PrEP users with high (73%) adherence and cost-saving in PrEP users with low (30%) adherence. It is also likely to be cost-saving in sex workers and serodiscordant couples not on PrEP, whether they have unmet need for contraception or are currently using oral contraception. DPP is unlikely to be cost-effective in oral contraceptive users ages 25–49 and would be net harmful if it reduced contraceptive adherence, depending on the setting. Results were robust to a range of time horizons or discount rates.

Conclusion: DPP is more likely to be cost-effective in settings and populations with higher HIV incidence. There is a risk that DPP could be net harmful for current oral contraceptive users if it led to substantial reductions in contraceptive adherence. DPP implementers should consider monitoring DPP adherence and promoting uptake in high-risk populations.

Cost-effectiveness of the dual prevention pill for contraception and HIV prophylaxis in Kenya and South Africa

<table>
<thead>
<tr>
<th>Population</th>
<th>DPP adherence</th>
<th>Comparison</th>
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</thead>
<tbody>
<tr>
<td>Ages 25–49</td>
<td>90%</td>
<td>10%</td>
</tr>
<tr>
<td>Sex worker</td>
<td>90%</td>
<td>10%</td>
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<tr>
<td>Serodiscordant</td>
<td>90%</td>
<td>10%</td>
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Table. Modelled effect of community TLD/PEP across settings scenarios in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Setting</th>
<th>Total</th>
<th>Cost-saving</th>
<th>Cost-effectiveness</th>
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<tr>
<td>SSA</td>
<td>84%</td>
<td>66%</td>
<td>73%</td>
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968 POTENTIAL IMPACT & COST-EFFECTIVENESS OF WIDELY ACCESSIBLE TLD IN SUB-SAHARAN AFRICA

Andrew Phillips1, Loveone Bansi-Matharu2, Maryam Shahmehni3, James Hargreaves4, Paul Reilly5, Euphemia Sibanda6, Jens Lundgren7, Catherine Godfrey8, Frances M. Cowan8, Valentina Cambiano1

1University College London, London, United Kingdom, 2Africa Health Research Institute, Durban, South Africa, 3London School of Hygiene & Tropical Medicine, London, United Kingdom, 4University of York, York, United Kingdom, 5Center for Sexual Health & HIV/AIDS Research, Harare, Zimbabwe, 6Hospital of Copenhagen, Copenhagen, Denmark, 7Massachusetts General Hospital, Boston, WA, USA, 8Imperial College London, London, United Kingdom

Background: Long-acting injectable cabotegravir (CAB-LA) demonstrated superiority to daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for HIV pre-exposure prophylaxis (PrEP) in two clinical trials. It is recommended by WHO as an additional prevention choice for people at substantial risk of HIV infection. HIV Modeling Consortium and HPTN Modeling Center conducted a comparative modelling analysis of the potential impact of expanding PrEP coverage by offering CAB-LA to men and women in South Africa.

Methods: Three independent age- and risk-stratified HIV transmission models (Synthesis, EMOD and Thembisa) were parameterised and calibrated to local data from South Africa. Expanding population PrEP coverage to 5 or 10% by 2050 was simulated by recruiting additional users based on model-specific targeting of PrEP use by risk. Models assumed 95% CAB-LA effectiveness based on HPTN 083 and model-specific TDF/FTC effectiveness. Population impact and efficiency of PrEP expansions were evaluated over 20 years compared to base-case scenarios assuming negligible TDF/FTC use.

Results: In the base-case scenarios with no PrEP expansion, median overall PrEP coverage in South Africa is currently < 1% and is modelled to remain < 1% by 2042. Achieving 5% PrEP coverage with CAB-LA by 2027 may avert 45% of new infections between 2022-2042 when use is assumed to be available to all
Results: Of the 367 (365 MSM) who started PrEP and contributed 1249 person-years of observation, the IRs for all STIs were 2.15 [1.95, 2.38] per 100 person-years. The IRs for syphilis were 0.07 [0.03, 0.14] per 100 person-years. The IRs for chlamydia and gonorrhea were 1.81 [1.64, 1.99] per 100 person-years. The IRs for HIV were 0.54 [0.31, 0.91] per 100 person-years for the non-STI group and 0.57 [0.32, 1.05] per 100 person-years for the STI group (p = 0.68). The IRs for hepatitis C (HCV) were 0.61 [0.35, 1.05] per 100 person-years, and the IRs for hepatitis B (HBV) were 0.05 [0.01, 0.21] per 100 person-years.

Conclusion: Our findings suggest that PrEP use is associated with a substantial reduction in incident STIs. Further studies are needed to assess the long-term impact of PrEP on HIV transmission and the potential for PrEP to reduce the burden of STIs in the general MSM population.
testing and treatment of STIs remain a priority among PrEP users. Biomedical prevention of STIs can be examined in this context.

Table: Characteristics of pregnant women randomized to point-of-care testing or standard, syndromic STI management in antenatal care, Cape Town, South Africa, 2012–2013

<table>
<thead>
<tr>
<th>Total (n=225)</th>
<th>Intervention: On-Demand point of care (n=113)</th>
<th>Standard: Syndromic STI management (n=112)</th>
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<tbody>
<tr>
<td>Gestational age (mean; median)</td>
<td>22 weeks (20-30)</td>
<td>22 weeks (20-30)</td>
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<td>Reported age (mean; median)</td>
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<td>Reported sexual orientation</td>
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<td>Reported rapid diagnosis</td>
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<td>29</td>
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<td>Reported mixed mean</td>
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<td>Reported missed clinic visit</td>
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<td>Reported missed visit</td>
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<td>Diagnosed with STI (WBC/CT)</td>
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<td>Diagnosed with bacterial infection</td>
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<td>Diagnosed with chlamydial infection</td>
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<td>Diagnosed with herpesvirus infection</td>
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972 PEP-IN-POCKET (PIP): LONG-TERM FOLLOW-UP OF ON DEMAND HIV POST-EXPOSURE PROPHYLAXIS

Maxime J. Billick1, Karla N. Fisher1, Samantha Myers1, Darrell H. S Tan4, Isaac I. Bogoch2

1University of Toronto, Toronto, ON, Canada, 2University Health Network, Toronto, ON, Canada, 3McMaster University, Toronto, ON, Canada, 4St Michael’s Hospital, Toronto, ON, Canada

Background: Pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) are two established methods to prevent HIV acquisition. However, the suitability of these tools for individuals with infrequent, higher-risk HIV exposures might be limited due to cost, high pill burden, or barriers to care. HIV post-exposure prophylaxis-in-pocket (PIP) involves prospectively identifying individuals with a low frequency of high-risk exposures and providing them with 28 days of PEP medication before an exposure occurs, along with instructions on when to initiate medications and how to follow up with care. We present long-term follow-up of a cohort of patients provided with PIP for HIV prevention.

Methods: We conducted a retrospective evaluation of the clinical characteristics and outcomes of patients who used PIP as their primary HIV prevention method. Patients referred for PrEP or PEP care were offered the option of PIP if they reported an ongoing risk of low frequency (0-4 per year), high-risk HIV exposures of any type. Importantly, HIV prevention method was chosen based on shared decision-making between patients and clinicians and was outside the realm of this study. Typical PIP regimen prescribed include Biktarvy® and tenofovir disoproxil fumarate/emtricitabine plus dolutegravir. Patients were followed at regular 4-6 months intervals. Demographic and clinical information was collected from two large HIV-prevention and care centers in Toronto, Canada between January 2016 and June 2022.

Results: PIP was prescribed to 109 patients, who were followed for a total of 168 patient-years. The average age was 37 years-old (range 20-69), with 106 (97.2%) patients assigned male at birth. Thirty-three (30.3%) patients self-initiated a total of 59 courses of PIP during the observation period. Patients fluidly transitioned between HIV prevention modalities as circumstances warranted: 34 (31.2%) changed from PIP to PrEP, and 32 individuals (29.4%) changed from PrEP to PIP. There were 13 episodes of bacterial sexually transmitted infections in 9 individuals (8.3%) using PIP. No HIV seroconversions were detected.

Conclusion: PIP is an innovative and useful HIV prevention modality for individuals with a low frequency of higher-risk HIV exposures. Patients may transition between PIP and PrEP based on evolving HIV risk. PIP may be included with PrEP and PrEP as a biomedical HIV prevention option for HIV-negative individuals at risk for infection.

973 ACCEPTABILITY AND FEASIBILITY OF A NEW URINE-BASED TENOFOVIR ADHERENCE TEST IN KENYA

Kenneth Ngure1, Phelix Okello2, Vallery Ogello2, Peter Mogere1, Stephen Gakuo2, Deepaliya Chakravarty3, Charlene Biwott4, Purba Chatterjee5, Jennifer Vellozo1, Nelly Mugo6, Monica Gandhi3

1Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, 2Kenyatta Medical Research Institute, Thika, Kenya, 3Partners in Health Research and Development, Thika, Kenya, 4University of California San Francisco, San Francisco, CA, USA, 5Kenyatta Medical Research Institute–UCSF Infectious Disease Research Training Program, Thika, Kenya, 6Kenyatta Medical Research Institute, Nairobi, Kenya

Background: Objective measures of oral pre-exposure prophylaxis (PrEP) adherence — especially point-of-care (POC) measures that enable real-time assessment, intervention, and feedback — have the potential to improve adherence. Our team previously developed and validated a novel urine-based POC measure of PrEP adherence. In this study, we sought to determine whether this urine assay for real-time adherence monitoring is acceptable and feasible among women taking PrEP and PrEP providers in Kenya.

Methods: We conducted semi-structured in-depth interviews with 17 women on PrEP who were enrolled in the POC assay arm of the PUMA trial (NCT03935464) and their seven providers in Thika, Kenya. Interviews were conducted after the 12-month study follow-up period. Interview guides explored acceptability, feasibility, and perceived benefits of and concerns about POC urine adherence testing for PrEP among participants and providers. Transcripts were analyzed using rapid qualitative analysis approaches with memo-writing and data tables used to synthesize key themes.

Results: Most participants reported that the POC test improved their PrEP adherence since they wanted to receive positive results. For example, one woman said, “I don’t skip drugs as [I did] before to avoid negative results”. Positive adherence results also led to less worry of acquiring HIV. Women liked that the POC test was clinic-based because subsequent counseling and interpretation of results was aided by clinic providers. The providers reported that having real-time adherence results enabled them to tailor counseling to individual needs, what some referred to as ‘evidence-based counseling’. Concerns expressed by participants included perception of lack of trust among providers and embarrassment associated with providing a urine sample. Provider concerns included the POC test not measuring long term adherence and potentially affecting retention of women with adherence challenges. The initial interpretation of results was challenging for providers, although they reported improvements with more familiarity with the test. Additionally, providers reported that the POC test would be more feasible if the kits were widely available and marketed for clinical use.

Conclusion: Our findings suggest that the POC urine TFV adherence test is highly acceptable and feasible for women on PrEP and their providers. Future studies should evaluate the impact of this novel test on adherence patterns over time in diverse populations.

974 WITHDRAWN
975 RANDOMIZED TRIAL OF DYNAMIC CHOICE PREVENTION AT OUTPATIENT DEPARTMENT IN EAST AFRICA

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SEARCH Study Team
1University of California San Francisco, San Francisco, CA, USA, 2Kenya Medical Research Institute, Kisumu, Kenya, 3Infectious Diseases Research Collaboration, Kampala, Uganda, 4University of California Berkeley, Berkeley, CA, USA, 5National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 6Kenya Medical Research Institute, Nairobi, Kenya, 7Makerere University, Kampala, Uganda

Background: Dynamic choice models for delivering HIV prevention may increase coverage for persons at risk. Data are limited on actual product choices made by clients and the impact of choice-based delivery models on prevention coverage. Outpatient departments (OPD) in health facilities in rural sub-Saharan Africa account for a high proportion of new HIV diagnoses, but are an understudied entry point to biomedical HIV prevention, including dynamic choice models.

Methods: We conducted an individually randomized trial of a dynamic choice HIV prevention (DCP) intervention vs. standard-of-care referral to prevention services (SOC) among adults with current or anticipated risk of HIV exposure seen at OPD clinics in rural Kenya and Uganda (SEARCH; NCT04810650). DCP included 1) product choice (daily oral PrEP [TFDF/FTC] or post-exposure prophylaxis [PEP]) with option to switch over time, 2) service location choice, 3) HIV self-testing option, 4) 24/7 phone access to clinician, and 5) provider training on client-centered care. Primary outcome over 48 weeks was biomedical covered time (proportion of follow-up covered by PrEP/PEP), assessed via self-report; secondary outcomes included coverage during periods of retrospectively self-assessed HIV risk.

Results: We enrolled 403 participants from April-July 2021, (197 DCP, 206 SOC); 61% women, 37% ages 15-24 years, 25% serodifferent partner, 88% HIV status unknown partner, 7% with prior PrEP or PEP use. In the DCP arm, 86% ever chose PrEP; 13% PEP over 48 weeks; selection of HIV self-testing increased from 26% to 51% and of out-of-facility visits from 8% to 52% during follow-up. Among 376/403 (93%) with outcomes ascertained, mean biomedical covered time was higher in DCP (47%) vs. SOC (18%); a difference of 29.2% (95% CI 22.7-35.7%; p< 0.001). Effect sizes were similar among men and women (28% and 31% higher coverage in the intervention arm, respectively). Intervention effect on coverage during periods at risk of HIV was larger; mean at-risk covered time was 65% in the DCP arm vs. 26% in SOC (difference 38.6%; 95%CI: 31.0-46.2%; p< 0.001).

Conclusion: In this randomized study, a dynamic choice prevention intervention with choice of PrEP/PEP, visit location, and HIV testing, plus client-centered care resulted in two-fold greater time covered by a biomedical prevention option compared to SOC among both men and women at elevated risk of HIV seen in a general outpatient department.

976 INCREASED UPTAKE OF BIOMEDICAL HIV PREVENTION BY YOUTH THROUGH COMMUNITY-BASED SRH

Maryam Shahmanesh1, Osee Behuhuma1, Kobus Herbst2, Jana Jarolimova2, Isisekelo Khoza1, Deenan Pillay3, Glory Chidumwa1, Natsayi Chimbindi1, Carina Herbst1, Nonhlanhla Okesola1, Jaco Dreyer1, Thembelihle Zuma1, Theresa Smit1, Jean-Michel Molina1

Isisekelo Sempilo Research Group
1Africa Health Research Institute, Durban, South Africa, 2Massachusetts General Hospital, Boston, MA, USA, 3University College London, London, United Kingdom, 4University of Paris Cité, Paris, France

Background: Despite free and efficacious biomedical HIV prevention, including universal HIV test and treat (UJT) and Pre-Exposure Prophylaxis (PrEP), 200,000 South Africans acquired HIV in 2019. Incidence was highest among adolescents and youth, so strategies to improve HIV prevention are needed.

Methods: We conducted a 2x2 factorial trial (Isisekelo Sempilo) between March 2020 and August 2022. 2301 eligible 16-29-year-olds, randomly selected from a population surveillance area in rural KwaZulu-Natal, were randomly allocated to four arms: 1) enhanced Standard of Care (SoC); access to study-organized mobile adolescent friendly services (AFYS) for differentiated HIV prevention (condoms, UJT, PrEP if eligible); 2) Sexual and Reproductive Health (SRH); baseline self-collected specimens for sexually transmitted infection (STI) testing and referral to AFYS for differentiated HIV prevention integrated with SRH; 3) Peer-support: referral to a peer navigator for needs assessment to tailor health and social support, condon provision and facilitation of AFYS attendance for differentiated HIV prevention; 4) SRH + peer-support. Co-primary outcomes:1) the proportion of individuals with transmissible HIV (HIV viral load >400 copies/ml) measured from dried blood spots (DBS) collected at 12 months; 2) linkage to study AFYS for differentiated HIV prevention and care within 60 days of enrolment.

Results: 1743 (76%) eligible contacted individuals were enrolled and randomised; 1168 (67%) provided a DBS at 12 months. Baseline characteristics and outcome ascertainment were similar by arm. At 12 months 227 (19%) tested ELISA-positive for HIV, of which 185 (82%) had a HIV viral load < 400 copies/ml. Overall 41 (3.5%) of all who provided DBS had transmissible HIV. After adjustment for age, sex and rural/urban area there was no difference in transmissible HIV by either intervention: SRH: aOR 1.12; 95%CI:0.6-2.11; peer-support aOR 1.03; 95%CI:0.55-1.94. Overall 732 (42.6%) linked to AFYS by 60 days. Those randomised to SRH were significantly more likely to link (aOR 1.6); 95%CI:1.32-1.95) but peer-support had no effect unless combined with SRH.

Conclusion: In this representative sample of adolescents and youth in rural South Africa, STI testing and SRH (but not peer support) increased uptake of differentiated HIV prevention. While the UNAIDS target of 90:90:90 was exceeded in all arms, neither SRH nor peer support reduced transmissible HIV compared to AFYS.

Time to linkage to AFYS for differentiated HIV prevention, including PrEP/ART amongst n=1743 16-29 year olds.
977 EFFECTS OF 6-MONTH PrEP DISPENSING WITH HIV SELF-TESTING ON SEXUAL BEHAVIORS IN KENYA
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Background: Six-month PrEP dispensing supported with interim HIV self-testing (HVST) was shown to be non-inferior compared to 3-month PrEP dispensing with clinic-based HIV testing, the standard of care (SOC), for PrEP continuation outcomes in Kenya. We assessed the effects of this differentiated service delivery (DSD) model for PrEP on sexual behaviors.

Methods: The JIPime-JiP-PrEP trial (NCT03593629) was a randomized non-inferiority implementation trial in Thika, Kenya among HIV-negative adults ≥18 years who had been using PrEP for 1 month. Participants were randomized 2:1 to: 1) 6-month PrEP dispensing with interim HVST and biannual clinic visits, or 2) SOC 3-month PrEP dispensing with clinic-based HIV testing and quarterly clinic visits. Participants reported the following sexual behaviors at every visit: any inconsistent condom use and number of sex partners, both in the past month. We conducted complete case analyses and used binomial regression models to estimate risk differences (RDs) for our binary outcome and linear regression models to estimate differences in means (DMs) for our continuous outcome. We adjusted all models for sex, HIV serodifferent partner status, and the corresponding baseline measure.

Results: From May 2018 to February 2020, we enrolled 959 participants (intervention: 329; SOC: 166), of whom 67% were women and 60% were in a serodifferent partnership. The median age was 33 years (interquartile range 27–40). Retention was similar across arms at both 6 months (intervention: 84.3%; SOC: 84.2%) and 12 months (intervention: 89.1%; SOC: 91.9%). Inconsistent condom use did not differ significantly between intervention and SOC arms at either 6 months (intervention: 90% vs. SOC: 87%; RD 3.1%, 95% CI -3.4%, 9.6%) or 12 months (intervention: 91% vs. SOC: 94%; RD -1.0%, 95% CI -5.8%, 3.8%). Additionally, the mean numbers of sex partners for participants were very similar in both arms at 6 months (intervention: 1.1 vs. SOC: 1.1; DM 0.0, 95% CI -0.3, 0.2) and 12 months (intervention: 1.0 vs. SOC: 1.0; DM 0.0, 95% CI -0.1, 0.2).

Conclusion: In this randomized implementation trial in Kenya, 6-month PrEP dispensing supported with interim HVST did not significantly change participants’ sexual behaviors compared to SOC 3-month PrEP dispensing with clinic-based HIV testing. These findings further emphasize that this DSD model of PrEP, featuring fewer follow-up clinic visits and interim home-based HVST, is safe, supporting its use to help simplify PrEP delivery in Kenya and similar settings.

978 PHARMACY-BASED PrEP DELIVERY IN KENYA: FINDINGS FROM A PILOT STUDY EXTENSION
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Background: To increase access to daily oral HIV pre-exposure prophylaxis (PrEP), policy makers and implementers are seeking evidence on whether and how PrEP can be delivered with high quality in non-clinical settings, including private pharmacies. Following a 12-month pilot study of pharmacy provider-initiated and managed PrEP, we conducted a 6-month extension to evaluate a modified version of this model that additionally offered post-exposure prophylaxis (PEP) and sexually transmitted infection (STI) testing.

Methods: We piloted the modified delivery model in 12 private pharmacies in Kisumu and Kiambu Counties. Pharmacy providers were trained to administer an HIV risk assessment screening tool; assess medical safety; assist clients with HIV self-testing; counsel; consult a remote clinician about complex cases and, if needed, refer to nearby public health facilities; and dispense. Eligible clients (≥18 years) received all services for free. Client and provider perceptions of model acceptability were assessed using Likert-type items derived from the Theoretical Framework of Acceptability.

Results: From January to July 2022, we screened 989 clients and initiated 863 (87%) on PrEP (n=684), PEP (n=173), and/or STI testing (n=53). Fig. 1. Among these 863 clients, 46% were male (n=400), 46% were <25 years (n=399), and 78% were unmarried (n=707). Most PrEP clients (n=684) reported inconsistent condom use (86%, n=587), not knowing partners’ HIV status (67%, n=459), and having multiple partners (62%, n=425). PrEP continuation at one month was 72% (95% CI [68%, 77%]), 71% (95% CI [67%, 76%]) and 68% (95% CI [64%, 72%]) at 6 months. STI positivity rate was 26% (14/53); the most prevalent STI was gonorrhoea (8/14). Model acceptability was high, with the majority (70-100%) of clients and providers reporting that they liked getting/delivering PrEP/PEP at the pharmacy and that getting/delivering PrEP/PEP at the pharmacy was not hard.

Conclusion: Our findings suggest that, when trained, pharmacy providers are capable of initiating and managing clients on PrEP in accordance with national guidelines and that additionally offering PEP and STI testing is a promising strategy for engaging clients in PrEP services. If this model was scaled up in Kenya, the rates of PrEP uptake and continuation might match or exceed those seen in public health facilities. More research is needed to identify business models (e.g., cost-sharing options) capable of sustaining pharmacy-delivered PrEP services in the long-term.

Figure 1. Study flow diagram
analyses, the urine assay was a significant predictor of DBS TFV-DP (OR = 14.1, p < .001); self-report did not add significantly to prediction.

**Conclusion:** The urine POC TFV assay had excellent predictive values and self-report did not add significantly to prediction of adherence. The POC assay provides results in several minutes to enable same-visit counseling; intervention, requires no specialized training, and is projected to be low-cost. It could also be used for research where objective short term adherence metrics are needed.

Positive and negative predictive values for PrEP adherence

<table>
<thead>
<tr>
<th>Drug</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/FTC</td>
<td>74%</td>
<td>99%</td>
</tr>
<tr>
<td>TAF/FTC</td>
<td>69%</td>
<td>99%</td>
</tr>
<tr>
<td>CAB-LA</td>
<td>60%</td>
<td>89%</td>
</tr>
</tbody>
</table>

980  **ORAL AND INJECTABLE PrEP USE IN THE UNITED STATES, 2013 TO 2022**

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**Background:** Three oral PrEP drugs have been approved by the FDA: branded tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) in July 2012, branded tenofovir alafenamide and emtricitabine (TAF/FTC) in October 2019, and generic TDF/FTC in July 2020. Long-acting injectable cabotegravir (CAB-LA) was approved in December 2021. We estimated trends in prescriptions for these PrEP drugs.

**Methods:** We analyzed IQVIA Real-World Data — Longitudinal Prescription Database (IQVIA) using a validated algorithm to identify persons prescribed antiretroviral drugs for PrEP. We estimated the number of persons prescribed branded TDF/FTC, TAF/FTC, generic TDF/FTC, or CAB-LA by month from January 2013 through June 2022. We estimated the proportions of prescriptions in June 2022 by type of PrEP drug. Among persons with an initial CAB-LA prescription from January through May 2022, we estimated the proportion who received a second prescription one month later. Among persons prescribed PrEP from January through June 2022, we estimated their demographic characteristics stratified by a prescription for oral PrEP or CAB-LA.

**Results:** We found that the number of persons prescribed branded TDF/FTC increased from January 2013 until October 2020 and then decreased markedly each month after TAF/FTC and generic TDF/FTC became available (Figure). Beginning in December 2021, the number of persons prescribed generic TDF/FTC exceeded the number prescribed TAF/FTC each month. In June 2022, 177,293 persons were prescribed PrEP: 89,654 (50.6%) were prescribed generic TDF/FTC and 80,754 (45.5%) TAF/FTC; only 804 (0.5%) were prescribed CAB-LA. From January through June 2022, we estimated the proportions of prescriptions in June 2022 by type of PrEP drug. Among persons with an initial CAB-LA prescription from January through May 2022, we estimated the proportion who received a second prescription one month later. Among persons prescribed PrEP from January through June 2022, we estimated their demographic characteristics stratified by a prescription for oral PrEP or CAB-LA.

**Conclusion:** Most PrEP users were prescribed generic TDF/FTC and very few were prescribed CAB-LA since its recent approval. The increasing proportion of generic TDF/FTC prescriptions compared with TAF/FTC is encouraging and can result in lower healthcare expenditures for PrEP; yet a large proportion of prescriptions were for the more expensive TAF/FTC. Better understanding is needed of reasons for low uptake of CAB-LA, including of operational barriers to its implementation. Studies are also needed to understand factors associated with CAB-LA use among women to inform implementation efforts.
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Background: There are limited quantitative studies describing the association between meth use in the context of male-male sexual partnerships and PrEP care engagement. We assessed the longitudinal relationship between individual and partnership level meth use with inconsistent PrEP engagement among young gay, bisexual and other men who have sex with men (GBMSM) in Los Angeles.

Methods: The mSTUDY (Men Who Have Sex with Men and Substance Use Cohort at UCLA Linking Infections, Noting Effects) is a cohort of GBMSM between the ages of 18 and 52 in Los Angeles. The primary outcome, PrEP engagement, had two levels. We defined consistent PrEP engagement as 18 consecutive months of reported PrEP care. Inconsistent PrEP engagement was defined by 6 months of PrEP care followed by either 12 months of non-engagement or 6 months of non-engagement and 6 months of engagement of the primary exposure was meth use at the partnership level with a ternary variable (neither partner nor participant used meth, either used meth, or both used meth). We measured sexual risk characteristics including STI test positivity. Generalized estimating equations were used to assess odds of inconsistent PrEP engagement at different levels of partner-participant meth use, adjusting for age at visit, number of recent male partners and partner intimacy.

Results: The sample included 149 unique participants (n=602 visits), of whom 48% were Black/African American, 36% were Hispanic/Latinx and 9% were white. There were 447 continuous PrEP events and 155 inconsistent PrEP events. Among inconsistent PrEP engagement, 61% (vs 75.5% continuous) reported that neither they nor their partner used meth, 22% (vs 18%) reported that either partner or participant used meth and 77% (vs 8%) reported that both partner and participant used meth (P < 0.01). Positive gonorrhea test was higher among inconsistent PrEP engagement (13.2%) vs. consistent (5.4%, P = 0.04). There were increased odds of inconsistent PrEP engagement when both partner and participant reported meth use (aOR: 3.82; 95% CI: 1.83-7.99) and when either partner or participant reported meth use (aOR: 2.46; 95% CI: 1.28-4.75) (Figure).

Conclusion: Meth use plays an important role in consistent PrEP engagement among GBMSM in mSTUDY. Odds of inconsistent PrEP engagement were higher when meth use was reported by either participant and partner or both. PrEP users who use meth with partners may benefit from integrated interventions addressing both meth use and PrEP engagement.

Adjusted odds of inconsistent PrEP engagement versus no meth use. Model also controls for age at visit, partnership assessment score and number of male partners.

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Background: The Ending the HIV Epidemic initiative aims to reduce new HIV infections by 90% by 2030. San Francisco is achieving progress toward this goal among men who have sex with men (MSM), with new HIV infections decreasing by 28% from 2019 to 2021. This period coincides with high and rising pre-exposure prophylaxis (PrEP) use in the last year, from 60% to 70%. Yet HIV infections among people who inject drugs (PWID) rose by 48% during this period, now accounting for 27% of new HIV diagnoses in San Francisco. In 2018, only 2.9% of PWID used PrEP in San Francisco. We examined PrEP cascade indicators among PWID in San Francisco, comparing data from 2018 to 2022.

Methods: Data originated from the National HIV Behavioral Surveillance (NHBS) study, which comprises serial cross-sectional surveys to assess HIV prevalence in key populations at risk in the US. Data were from PWID, who resided in San Francisco between June and December of 2018 and 2022. Respondent-driven sampling was used for recruitment. This analysis compared PrEP cascade indicators from 2018 to 2022 among PWID who self-identified as HIV-negative or unknown.

Results: Of 479 HIV-negative/unknown PWID, more than half had a usual source of care (76.6%) and healthcare visits in the past 12 months (75.3%). Only 54.9% were aware of PrEP, 5.9% discussed PrEP with healthcare providers (HCP), and 1.5% used PrEP in the past 12 months. PrEP indicator estimates were comparable to or significantly worse than those of 2018: 54.1% (p = 0.796), 12.9% (p < 0.001), and 2.9% (p = 0.003), respectively. Factors associated with low PrEP awareness among PWID in 2022 were Black/African American race/ethnicity (OR 0.50 vs. Whites, p = 0.001, 95% CI 0.33-0.76), household income below the federal poverty level (OR 0.35 vs. above, p = 0.017, 95% CI 0.15-0.83), and not testing for HIV (OR 0.43 vs. testing, p = 0.001, 95% CI 0.30-0.63), HCV (OR 0.48 vs. testing, p < 0.001, 95% CI 0.33-0.70) or sexually transmitted diseases (STDs) (OR 0.44 vs. testing, p < 0.001, 95% CI 0.30-0.66) in the past 12 months.

Conclusion: PrEP discussions with HCP and PrEP use were significantly lower among PWID in 2022. Lack of PrEP awareness was associated with social determinants of race/ethnicity, income, and sexual health testing. Public health interventions to increase HIV testing and PrEP discussions from HCP to PWID may have the greatest potential to improve PrEP awareness and use among PWID, particularly among Black/African American residents and PWID with low income.

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1University of Washington, Seattle, WA, USA, 2Infectious Diseases Institute, Kampala, Uganda, 3Infectious Diseases Institute, Kampala, Uganda, 4RTI International, San Francisco, CA, USA, 5Harvard Medical School, Boston, MA, USA, 6Makerere University, Kampala, Uganda, 7Makerere University, Kampala, Uganda, 8University of Alabama at Birmingham, Birmingham, AL, USA

Background: Experiences of intimate partner violence (IPV) are associated with reduced adherence to antiretroviral (ART) and pre-exposure prophylaxis (PrEP) nonadherence, increased risk of HIV acquisition and poorer engagement in HIV care. There is limited evidence describing the ways in which exposure to violence and other maladaptive relationship dynamics may influence ART and PrEP adherence for both individuals in committed serodiscordant partnerships.

Methods: Using longitudinal data from a stepped-wedge cluster randomized trial, we evaluated the effect of IPV exposure on ART and PrEP adherence for both partners in serodifferent unions. The primary outcome was PrEP or ART adherence—defined as either having a variable determined via self-reported or, if available, quantified tenofovir disoproxil fumarate (for PrEP) or HIV viral load (for ART) biomarker data. The primary predictor, IPV exposure, was evaluated using a modified Conflict Tactics Scale to identify exposure to physical or sexual IPV since the last visit. As a secondary predictor, we used the Sexual Relationship Power Scale to assess perceived relationship dynamics. Outcome and predictor data were evaluated at 1-month and quarterly visits. We used generalized
estimating equations to assess the association between IPV exposure and ART/PrEP adherence.

**Results:** From June 2018 to December 2020, we enrolled and followed both partners in 149 heterosexual serodifferent couples. The median age at enrollment was 28 years (IQR: 24 - 33). The partner living with HIV was female in 65% of couples. At enrollment 19% of male and 20% of female partners reported having experienced sexual or physical IPV in the past year. Recent IPV exposure was not significantly predictive of PrEP or ART adherence (OR: 1.27, 95% CI 0.52 – 3.16). Compared to their counterparts living with HIV, IPV-exposed HIV-negative partners had similar levels of PrEP adherence (OR: 0.77, 95% CI 0.19 – 3.14). Compared to their counterparts living with HIV, HRT partners who reported more imbalanced relationship power dynamics had odds trending towards higher adherence (OR: 1.06, 95% CI 0.99 – 1.13).

**Conclusion:** We found that IPV was not predictive of adherence to PrEP or ART. These findings contribute to the evidence base outlining the association between IPV and ART/PrEP adherence in HIV serodifferent couples. Future research should leverage event-level analyses to further evaluate the circumstances and manners in which distinct types of IPV influence HIV treatment and prevention outcomes.

**985 DETERMINANTS OF PRE-EXPOSURE PROPHYLAXIS RETENTION AMONG TRANSGENDER WOMEN**

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**Background:** Transgender women (TW) face unique challenges staying engaged with HIV prevention services, including pre-exposure prophylaxis (PrEP). To inform efforts to increase PrEP retention among TW, we examined barriers and facilitators of PrEP retention among TW in Southern California.

**Methods:** We used a mixed methods sequential explanatory approach with 2 phases. In Phase 1, we used quantitative data from a PrEP demonstration project (Project EPT) that included PrEP provision among 170 TW to evaluate 24-week retention by sociodemographics, engagement in sex work, substance use, and hormone replacement therapy (HRT) use. In Phase 2, we conducted 15 in-depth interviews with PrEP experienced TW purposively sampled to include TW who engaged in sex work based on Phase 1 findings. We then used thematic analysis to explain Phase 1 findings and integrated them in the presentation of Phase 2 themes.

**Results:** In Phase 1, a greater proportion of participants who were not retained at 24 weeks reported engaging in sex work than those who were retained (18% vs 7%). In Phase 2, 2 subcategories of sex work engagement emerged. The first was characterized as engaging in “non-survival sex work” — these TW had little difficulty staying in PrEP care, sought clients from online sources, had stable housing, accessed HRT through providers, and exchanged sex primarily for money. The second was characterized as engaging in “survival sex work” — these TW struggled to stay in PrEP care, had street-based clients, were unstably housed, used black market hormones, and more frequently exchanged sex for drugs. In Phase 1, a smaller proportion of participants not retained at 24 weeks reported HRT than those who were retained (56% vs 71%). In Phase 2, participants consistently prioritized HRT over PrEP, describing HRT as a basic necessity and life-saving, yet many also described the bidirectional benefits of accessing HRT and PrEP. In Phase 1, a greater proportion of participants who were not retained at 24 weeks reported substantial or severe drug use than those who were retained (18% vs 8%). In Phase 2, participants reported drug use as a barrier to PrEP, often in the context of sex work.

**Conclusion:** TW who engage in “survival sex work” experience barriers to PrEP retention (e.g., unstable housing, drug use) which may require supportive resources to stay engaged with PrEP care. The reciprocal benefits of HRT and PrEP suggest that co-location of services may be an optimal strategy for enhancing PrEP retention among TW.

**Table 1. Sociodemographics of in-depth interview participants (N = 10).**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female/woman, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/men</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Trans female/trans woman, N (%)</td>
<td>7 (77)</td>
</tr>
<tr>
<td>Transman, male to female, transgender, or transsexual woman, N (%)</td>
<td>4 (77)</td>
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<tr>
<td>Race and ethnicity</td>
<td></td>
</tr>
<tr>
<td>Black or African American, N (%)</td>
<td>3 (100)</td>
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<tr>
<td>Latina/a, N (%)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>White, N (%)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Spanish or multiracial, N (%)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Currently on PrEP, N (%)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Sex work experienced, N (%)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Age, mean (standard deviation)</td>
<td>35.8 (9.7)</td>
</tr>
</tbody>
</table>
987 BONE DENSITY CHANGES WITH CAB-LA OR TDF/FTC PrEP IN MSM AND TGW IN HPTN 083

Todd T. Brown1, Robert Arao2, Nittaya Phanuphak3, Esper Kallas4, Tomitope Oyedele5, Richard Berman6, Philip Sullivan7, Brett Hancorn8, Adeola Adefuye9, Jim Rooney9, Alex R. Rinehart9, Andrea Jennings9, Marybeth McCauley9, Beatriz Grinsztejn11, Raphael J. Landovitz12

HPTN 083 Study Team
1The Johns Hopkins University, Baltimore, MD, USA, 2Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 3Institute of HIV Research and Innovation, Bangkok, Thailand, 4University of São Paulo, São Paulo, Brazil, 5Cook County Health, Chicago, IL, USA, 6Statistical Center for HIV/AIDS Research and Prevention, Seattle, WA, USA, 7Centers for Disease Control and Prevention, Abuja, Nigeria, 8Gilead Sciences, Inc, Foster City, CA, USA, 9HIV Healthcare, Research Triangle Park, NC, USA, 10FHI 360, Durham, NC, USA, 11Instituto de Pesquisa Clínica Evandro Chagas, Rio de Janeiro, Brazil, 12University of California Los Angeles, Los Angeles, CA, USA

Background: Bone mineral density (BMD) loss has been a concern for HIV pre-exposure prophylaxis (PrEP) containing tenofovir disoproxil fumarate (TDF). Long-acting cabotegravir (CAB-LA) was superior to TDF/emtricitabine (FTC) for HIV prevention in two clinical trials, but the relative bone safety of these regimens is unknown.

Methods: HPTN 083 conducted a bone safety sub-study to compare BMD changes over 105 weeks with CAB-LA and TDF/FTC in cisgender men who have sex with men (MSM) and transgender women (TGW) at risk for HIV infection from 22 international sites. BMD was measured at the lumbar spine (LS), femoral neck, and total hip by dual-energy x-ray absorptiometry (DXA) at baseline, 57 weeks, and 105 weeks. Percentage BMD change at each anatomic site was compared between the two randomized arms by two-sample, independent t-tests in those who received at least 10 bi-monthly injections over 18 months from enrollment (n = 254).

Results: At baseline the median (Q1, Q3) age was 27 (23, 35) years, 9.4% TGW, and 47.2% white. The proportion of those with low BMD at baseline (Z-score at any anatomic site ≤ -2.0) was 15%. At the LS, the median percentage change in BMD was 0.82% in the CAB-LA arm and -0.82% in the TDF/FTC arm (between arm difference 95% confidence interval [-1.6%, -0.87%], p < 0.01) at 57 weeks (n = 248). This difference persisted at 105 weeks (n = 203) with a between-arm difference in percentage BMD change of -2.3% (95% CI: -3.4%, -1.1%), p < 0.01. Similar results were observed at both the femoral neck and total hip (Figure). Conclusion: Among MSM and TGW receiving CAB-LA versus TDF/FTC HIV PrEP, bone mineral density trajectories over two years were different, with BMD gain in the CAB-LA arm and BMD loss in the TDF/FTC arm. For individuals with low BMD or other fracture risk factors, CAB-LA PrEP may confer benefits related to bone health compared to TDF-containing PrEP.

Percent BMD Difference From Enrollment by Study Arm

988 PHASE 1 TRIAL OF CAP256V2LS AND VRC07-523LS ANTIBODIES AMONG WOMEN IN SOUTH AFRICA

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Background: Young women in sub-Saharan Africa continue to bear a high burden of HIV infection. Combination anti-HIV monoclonal antibodies are a potential HIV prevention technology that may overcome adherence challenges of daily oral pre-exposure prophylaxis. CAPRISA 012B, a first-in-human dose-escalation phase 1 trial evaluated the safety, pharmacokinetics and neutralization activity of the monoclonal antibody CAP256V2LS alone and in combination with VRC07-523LS in young HIV-negative women in Durban, South Africa.

Methods: From 13 July 2020 to 13 January 2021, 42 healthy women, aged 18–45 years, were enrolled. Groups 1 and 2 were open-label and CAP256V2LS was administered at 5mg/kg and 10mg/kg intravenously; and 5mg/kg, 10mg/kg and 20mg/kg subcutaneously. In Group 3 participants were randomized to receive a combination of CAP256V2LS and VRC07-523LS at 10mg/kg and 20mg/kg subcutaneously co-mixed with a recombinant human hyaluronidase. Neutralizing activity was measured using env-pseudotyped viral particles in the TZM-bl assay. A quantitative electrochemiluminescence sandwich immunoassay was performed to determine antibody concentrations.

Results: There were no serious adverse events or dose-limiting toxicities. Most commonly reported symptoms following intravenous administration were headaches [7/8 (88%)] and nausea [4/8 (50%)]. Commonly reported symptoms following subcutaneous administration were headaches [31/34 (91%), chills [25/34 (74%)] and malaise/fatigue [19/34 (56%)]. Adverse events included transient lymphocytopenia [8/42 (19%)], proteinuria [9/42 (21%), elevated aspartate aminotransferase [10/42 (24%)] and alanine aminotransferase [5/42 (12%)]. At 6 months, the median serum concentration of CAP256V2LS and VRC07-523LS in participants who received 20mg/kg, was 6μg/mL and 26μg/mL, respectively. Overall, the estimated half-life was 43 days for CAP256V2LS and 66 days for VRC07-523LS. Neutralization data showed that both antibodies retained their functional activity post-administration. Conclusion: CAP256V2LS and VRC07-523LS administered subcutaneously alone and in combination with recombinant hyaluronidase was safe and well tolerated, with detectable antibody concentrations up to 6 months post administration. CAP256V2LS is one of the most potent antibodies described to date and in combination with VRC07-523LS is predicted to provide significant coverage of global HIV strains. These data support further assessment in larger clinical studies.

Figure 1: Median concentrations of CAP256V2LS and VRC07-523LS through 168 days post study product administration observed in the CAPRISA 012B trial.
VAGINAL PreP EFFICACY OF BIODEGRADABLE ISLATRAVIR IMPLANTS IN MACAQUES

Michele B. Daly1, Daniel Kim1, Linying Li1, Archanha Krovi1, Chasity Norton1, Catalina Forero1, Marie Brake1, Chuong Dinh1, Tiancheng Edwards1, Mackenzie Cottrell1, Walid Heneine1, Gerardo Garcia-Lerma1, Charles Dobard1, James Mitchell1, Leah Johnson2, Small sample size, efficacy and 95% confidence intervals were calculated with 100% efficacy. Median [range] plasma ISL levels during SHIV challenges were 25.4 [23.0- 28.4] and 5.2 [2.3-6.6] nM, respectively. All macaques with ISL-705 implants had adverse implant-site reactions (Draize score 1-3) that persisted for 8 weeks, at which point the implants were removed. In contrast, no implant-site reactions were observed in animals with two ISL-83 implants over the 12-week study period (144 observations). Macaques with two ISL-83 implants were exposed vaginally to SHIV162P3 twice-weekly at weeks 5-11 post-implantation (12 total challenges). Infection outcome was compared with 6 untreated animals (2 real-time and 4 historical controls). Blood was collected at each challenge to monitor plasma ISL and SHIV RNA.

Results: ISL-705 and ISL-83 implants reached steady-state by day 21 with median [range] plasma ISL levels of 25.4 [23.0- 28.4] and 5.2 [2.3-6.6] nM, respectively. All macaques with ISL-705 implants had adverse implant-site reactions (Draize score 1-3) that persisted for 8 weeks, at which point the implants were removed. In contrast, no implant-site reactions were observed in animals with two ISL-83 implants (n=12) over the 12-week study period (144 observations). Macaques with two ISL-83 implants (176 µg/d) remained uninfected during a cumulative of 72 SHIV exposures, while the 6 controls were infected after a median of 2.5 challenges (range 1-5) resulting in 100% efficacy. Median [range] plasma ISL levels during SHIV challenges were 4.0 nM [1.2-7.0].

Conclusion: We show that release rates from ISL-705 implants may impact local toxicity. In contrast, we identified ISL-eluting implants (ISL-83) with a cumulative in vitro release rate of 176 µg/d that did not result in implant-site reactions and fully protected macaques against repeated vaginal SHIV exposures. We also defined clinically relevant plasma concentrations of 1-7 nM that were associated with complete vaginal protection. These data will inform the selection of safe and effective biodegradable ISL implants for HIV LA-PreP in women.

EXTENDED POST-EXPOSURE PROTECTION AGAINST SHIV VAGINAL INFECTION WITH TAF/EVG INSERTS

Natasha Makarova1, Dyana Singletary1, Missy Peet1, James Mitchell1, Angela Holder1, Chuong Dinh1, Yi Pan1, Maria Corazon Bueno Mendoza1, Meredith Clark2, Walid Heneine1, James Smith1, Gerardo Garcia-Lerma1, Gustavo F. Doncel2.

Background: On-demand HIV prevention modalities used before or after vaginal sex may be a desirable alternative to daily oral pre-exposure prophylaxis (PrEP) for women with infrequent or clustered sexual activity. CONRAD has developed inserts containing tenofovir alafenamide fumarate (TAF) and elvitegravir (EVI) that were safe, well accepted, and showed good pharmacokinetics and pharmacodynamics in a clinical trial (CONRAD A18-146) after vaginal administration. We recently showed that TAF/EVI inserts fully protected macaques against repeated SHIV vaginal exposures when administered as post-exposure prophylaxis (PrEP) 4h after virus exposure. Here, we sought to define the window of PEP activity by applying inserts 8 or 24 hours after SHIV exposure.

Methods: Cycling pitgaited macaques were challenged with low-dose SHIV162p3 and dosed 8 or 24h later with a TAF/EVI (20 mg/16 mg) insert (n=6 per group) or placebo insert. Animals were challenged weekly for 13 weeks. Infection was monitored by plasma virus load using RT-qPCR. Due to the small sample size, efficacy and 95% confidence intervals were calculated with Fisher’s exact test. Survival analysis was conducted to compare time to infection in 8h and 24h treated arms relative to 9 placebo controls (n=2 for 8 and 24h, n=5 historical controls) using the Log-Rank test (LRT) in SAS Proc Lifetest (SAS 9.4).

Results: At 8h, only 1/6 treated animals became infected (at exposure 11), resulting in high calculated efficacy of 94.41% (95% exact CI =57.03%, 99.27%) and a significant difference in the time to infection compared to placebo controls (p=0.0063, LRT). Extending the window of PEP to 24h resulted in 3/6 animals becoming infected at exposures 2, 2, and 13, thus decreasing the efficacy to 77.23% (95% exact CI =20.00%, 93.52%) and leading to the loss of significance in time to infection when compared to the controls. The median time to infection for the control group was 3 challenges.

Conclusion: These data extend the window of high post-exposure protection by a single TAF/EVI insert to 8h and define a reduction in efficacy at 24h post-exposure. The study supports the clinical development of the TAF/EVI insert as an on-demand PEP option for women and informs its dosing modality.

PHARMACOKINETICS AND XRAY IMAGING OF LONG-ACTING CABOTEGRAVIR IN SITU FORMING IMPLANT

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Background: Long-acting injectables (LAIs) for HIV PrEP can increase adherence and reduce stigma. Current LAIs are not removable and present a pharmacokinetic (PK) tail. To overcome these limitations, we developed an ultra-long-acting (ULA) biodegradable and removable in situ forming implant (ISFI) with cabotegravir (CAB). Here, we performed a PK study evaluating injection volume (IVOL), drug concentration in plasma and tissues, PK tail, and implant visualization with full-body x-ray imaging (FBXI).

Methods: PLGA-based ISFIs were comprised of 500 mg/mL CAB in a stable suspension. Female BALB/c mice (n=6/IVOL) were injected subcutaneously (SC) with 50, 100, or 2*50 µL of CAB ISFI formulation. Plasma, and vaginal, cervical, and rectal tissues samples were collected longitudinally to quantify drug concentration over ≥180 days. At day 30, 60, 90, and 180 post injection, depots were removed via a small skin incision to quantify residual CAB and determine PLGA molecular weight (MW) decrease over time. Plasma samples were collected 30 days post-depot removal to assess the PK tail. Additional CAB ISFI formulations were made with 10 wt% barium sulfate (BaSO4) to generate a radiopaque implant. Mice (n=3) were injected with 50 µL of CAB/BaSO4 ISFI (right flank) and 50 µL of placebo/BaSO4 ISFI (left flank). Mice underwent biweekly FBXI to assess implant visualization post-injection.

Results: ULA CAB ISFIs elicited concentration in plasma for all IVOLS well above the 4× PA-IC90 for 240 days (Fig A) and high tissue concentrations for 180 days (Fig B-D). CAB concentration in all matrices was not significantly different across IVOLS. Significant decrease in plasma CAB was achieved post depot removal with ~9-, 13-, 29-, and 11-fold decrease for depots removed at d30, 60, 90, and 180 respectively (Fig E-H), however, complete elimination of CAB was not achieved 30 days post depot removal, likely a result of CAB accumulation in the SC tissue and/or incomplete depot removal. ISFIs were retrievable after 180 days post-injection (Fig J) with ~25% CAB remaining and ~85% decrease in PLGA MW for all IVOLS. ISFIs with BaSO4 maintained visualization via FBXI for 86 days (Fig J).

Conclusion: ULA CAB ISFI is the first injectable for HIV PrEP that is biodegradable and removable, only if needed, elicited plasma and tissue PK for ≥180 days and is visualized for several months by FBXI. This comprehensive study further characterized the formulation and future directions include similar studies in non-human primates.
**LENACAPAVIR PROTECTS AGAINST RECTAL SHIV ACQUISITION IN MACAQUE MODEL**

Elena Bekerman, Stephen Yant, Laurie A. Vanderveen, Derek Hansen, Bing Lu, William Rowe, Kelly Wang, Christian Callebaut

**Background:** Pre-exposure prophylaxis (PrEP) is an important strategy for HIV prevention among people at risk. Long-acting antiretroviral agents circumvent the requirement for daily dosing to achieve maximal protection and represent an alternative to daily oral regimens. Lenacapavir (LEN) is a long-acting HIV capsid inhibitor recently approved in the European Union for the treatment of multi-drug-resistant HIV infection in combination with other antiretrovirals. Here, we assessed the relationship between LEN plasma concentration following a single subcutaneous (SC) administration and PrEP efficacy using a single high-dose SHIV rectal challenge macaque model.

**Methods:** LEN antiviral activity against SHIV and HIV was measured in activated PBMCs and adjusted for plasma protein binding. The SHIV stock was titrated over 8 cycles of escalating (0.625 to 100 TCID50) rectal challenge (n=8 macaques/cycle) to define a suitable high-dose inoculum. Twenty naïve rhesus macaques received a single SC injection of LEN at 5, 10, 20, 50 or 75 mg/kg (n=4/group). Eleven animals with LEN exposures above its paEC95 were challenged rectally with SHIV 7 weeks post-dose. Blood was collected weekly through study week 25 for the evaluation of drug levels, viral loads, and p27 ELISA.

**Results:** LEN inhibited SHIV replication in rhesus PBMCs with an EC50 of 390 pM, resulting in a projected 4.4-fold lower LEN activity against SHIV in vivo in rhesus as compared to HIV in humans (rhesus and human PBMC paEC95 values of 8.8 and 2.0 nM, respectively). A SHIV inoculum resulting in 10 infections out of 16 rectal challenges of untreated macaques (62.5% infection per challenge) was selected for the LEN efficacy study. Animals dosed with LEN exhibited a dose proportional increase in plasma Cmax and AUC and a half-life ranging from 17-53 days. Of 11 SHIV-challenged animals, 3 became infected and 8 remained protected as confirmed by plasma PCR, cell-associated proviral DNA assay, and serology. Adjusting for the 4.4-fold LEN potency difference between HIV and SHIV, we computed protection above and below the clinical efficacy target LEN plasma concentration in this model (70 nM). In animals with LEN above this target, LEN demonstrated complete protection and was superior to the untreated group (p=0.012, one-tailed Fisher’s exact test).

**Conclusion:** These data demonstrate effective SHIV prophylaxis in a stringent macaque model at clinically relevant LEN exposures and support the ongoing clinical evaluation of long-acting LEN for HIV PrEP.

**PRE-EXPOSURE PROPHYLAXIS PRODUCT CHOICE IN US PARTICIPANTS IN HPTN 083**

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**Background:** HPTN 083 demonstrated superiority of long-acting injectable cabotegravir (CAB-LA) compared to daily oral tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) for HIV pre-exposure prophylaxis (PrEP) in cisgender men and transgender women (TGW) who have sex with men. At a planned interim review, an independent data safety and monitoring board recommended the study be unblinded. The protocol was then amended to continue as an open-label extension (OLE) in which participants without contraindication were offered the choice of open-label CAB-LA or to complete study participation with daily oral TDF/FTC. United States (US) participants with daily oral TDF/FTC.

**Conclusion:** This is a retrospective cohort study of HIV negative female sex workers who were exposed or not exposed to PrEP in 22 out of 59 health facilities of the city of Kigali. We included eligible HIV negative FSWs aged 18 years and older who consented to taking PrEP for life (exposed) and compare those to whom they did not consent to taking PrEP (unexposed). Data were extracted in a csv format for analysis from an anonymized electronic database. We built logistic regression model the risk of HIV seroconversion at twelve months of follow-up after weighting the inverse probability treatment using a propensity score to control for confounding and reduce imbalance across different treatments.

**Results:** From January 2019 to October 2021, 2,544 HIV-negative FSW were followed-up in PrEP providing facilities. The majority of FSW 51.9% (1,322/2,544) was aged 18-29 years, 63.0% (1,604/2,544) lived alone and 73% (1,856/2,544) had only attended basic school. In total, 45.1% (1,147/2,544) of FSW participated in the PrEP program while 54.9% (1,397/2,544) were not. At 12 months of follow-up 78.9% (906/1,147) and 63.7% (890/1,397) of FSW were retained in PrEP and in the HIV prevention program, respectively. At 12 months’ follow-up, 0.56% (5/906) of FSW in the PrEP program and 1.69% (15/905) of FSW in program without PrEP became HIV-positive. We estimated 69% lower risk of HIV infection among FSW actively followed-up at 12 months’ participation in the PrEP program compared to participation in the pre-existing prevention program (adjusted odds ratio 0.31, 95% confidence interval: 0.11-0.87).

**Conclusion:** In this pilot study incidence of HIV infection at 12 months was lower in FSW who were retained in the PrEP program compared to FSW who were ineligible or did not consent to participate. We recommend further longitudinal studies to estimate patients’ long-term outcomes and scale-up to other high risk groups.
Results: Compared to control arm, all PrEP dosing arms significantly decreased p24 levels after ex vivo HIV-1 ι, challenge of foreskin tissue (all p < 0.0001) and PBMCs (p = 0.02 to < 0.0001) with no differences between drugs (TDF-FTC or TAF-FTC) or timings observed. Ex vivo PEP dosing did not increase protection in tissue but further reduced p24 levels in PBMCs. There was no difference in time to, or duration of protection between PBMCs and explants. Higher levels of TFVdp were obtained with TAF-FTC than TDF-FTC in foreskin and PBMCs (p < 0.0001). Foreskin CD4+ cell TFVdp levels were 42.7 (10.9) fmol/10^5 cells (TAF:DFC = p = 0.9). PrEP protection was lost with dosing after 48h exposure; TAF-FTC was significantly more protective than TDF-FTC at every time point. Tight junction proteins were not significantly affected. TDF-FTC significantly reduced IL-1a levels in foreskin.

Conclusion: Oral on demand PrEP with TDF-FTC or TAF-FTC provides ex vivo protection of foreskin and PBMCs. TAF-FTC provided higher TFVdp levels in foreskin and PBMCs, and better PrEP activity than TDF-FTC in foreskin. PrEP protection decayed after 48h post exposure.

996 INTERACTION BETWEEN TAF-BASED PREP AND HORMONE THERAPY IN TRANSGENDER WOMEN: iFACT3
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iFACT3 Study Team
1Chulalongkorn University, Bangkok, Thailand, 2Institute of HIV Research and Innovation, Bangkok, Thailand, 3Chuang Mai University, Chuang Mai, Thailand

Background: Feminizing hormone therapy (FHT) is common among transgender women (TGW) receiving PrEP. To evaluate the potential drug-drug interaction (DDI) between FHT and emtricitabine/tenofovir alafenamide (FTC/TAF)-based PrEP, we assessed the pharmacokinetics (PK) of FTC, TAF, tenofovir (TFV) and estradiol (E2) among TGW receiving FHT in Thailand.

Methods: Twenty TGW who had not undergone orchietomy and had not received injectable FHT within 3 months were enrolled between January and February 2022. FHT (estradiol valerate 2 mg and cyproterone acetate 25 mg) was prescribed to participants at baseline until week 9, while PrEP (TDF FTIC 200 mg/TFV 25 mg) was initiated at week 3 until week 12. Intensive PK sampling was performed at weeks 3 (FHT only) and 9 (PrEP and FHT) for E2; and weeks 9 (PrEP and FHT) and 12 (PrEP only) for plasma FTC, TAF, and TFV.

Blood bioavailable testosterone, FSH, and LH were also measured.

Results: 18/20 participants completed the PK visits and were included in this analysis. Median (interquartile range [IQR]) age and body mass index were 28 (23-32) years and 20.8 (19.9-21.9) kg/m^2, respectively. The area under the curve (AUC) and maximum concentration (Cmax) geometric mean ratios (GMRs) (90%CI) at week 3 (reference) and week 9 for E2 were 0.80 (0.72-0.90) p = 0.002 and 1.11 (0.94-1.31), p = 0.23, respectively. The AUC and Cmax GMRs at week 9 and week 12 (reference) were as follows: FTC, 0.92 (0.88-0.97, p = 0.009) and 0.93 (0.84-1.03, p = 0.24); TAF, 1.05 (0.83-1.33, p = 0.73) and 1.14 (0.85-1.52, p = 0.46); and TFV, 0.92 (0.88-0.97, p = 0.01) and 0.97 (0.89-1.05, p = 0.50) (Figure). No significant changes in bioavailable testosterone, FSH, and LH between weeks 3 and 9 were observed (bioavailable testosterone, median [IQR] 0.031 [0.025-0.120] vs 0.024 [0.016-0.122], p = 0.17; FSH, 0.75 [0.6-1.3] vs 0.85 [0.4-1.6], p = 0.24; and LH, 0.52 [0.21-0.86] vs 0.44 [0.25-0.79], p = 0.95. No participants discontinued the study due to a reported adverse event. There were no significant changes in creatinine clearance and alanine aminotransferase levels over the study period.

Conclusion: Plasma E2, FTC and TFV exposures trended lower when FTC/TAF was administered with FHT; however, the AUC and Cmax GMRs of FTC and TFV were within the bioequivalence range, indicating no clinically significant DDI from FHT towards FTC/TAF-based PrEP. Intracellular and tissue rectal measurements of TFV-TP and FTC-TP levels are ongoing.
PrEP USE AMONG US VETERANS USING VETERAN HEALTH ADMINISTRATION SERVICES: 2017-2021
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Background: The number of persons prescribed HIV preexposure prophylaxis (PrEP) in the United States has been estimated at the national, state, and county-levels using a large commercial pharmacy database. These data are used to calculate a quarterly PrEP coverage indicator to monitor progress towards the goals of the Ending the HIV Epidemic in the U.S. (EHE) initiative. This pharmacy database includes prescriptions from >90% of the U.S. retail pharmacies, but PrEP prescriptions from the Veteran Health Administration (VHA) system were not included. To address this gap, we analyzed VHA data to examine the trends in PrEP use among veterans receiving health services in the VHA system.

Methods: We analyzed 2017-2021 PrEP prescription data extracted from the VHA system. We reported the total annual number of persons aged ≥18 years prescribed PrEP each year, stratified by sex, age, race/ethnicity, and region. We assessed trends in the number of persons prescribed PrEP by calculating estimated annual percent change (EAPC) and 95% confidence intervals (CIs) using Poisson models.

Results: The number of veterans prescribed PrEP increased from 1,910 in 2017 to 4,847 in 2021 (EAPC: 24.0% [95% CI, 19.0–29.3]). In 2021, 4,847 veterans had PrEP prescriptions and 96.0% were men, 54.1% were aged 25─44 years, 52.3% were white, 24.6% Black/African American (Black) and 13.4% Hispanic or Latino. Majority of the 2021 PrEP prescriptions were in the South (42.5%), 1,676 (34.7%) in the West, 620 (12.8%) in the Midwest, and 486 (10.1%) in the Northeast. The number of male veterans prescribed PrEP increased during 2017-2021 with an EAPC of 29.3% and the proportion of Black veterans prescribed PrEP increased with an EAPC of 29.0% and the proportion of women who comprised PrEP users increased from 3.2% in 2017 to 4.7% in 2021. Similarly, the number of female veterans prescribed PrEP each year, stratified by sex, age, race/ethnicity, and region.

Conclusion: VHA data fill a gap in monitoring PrEP use in the United States. We observed an increasing trend in the number of veterans prescribed PrEP similar to the trends among persons with commercial or public health insurance. An increasing proportion of Black veterans prescribed PrEP over the study period can help to decrease racial/ethnic disparities in receipt of HIV prevention services. Less than 5% of PrEP users were female veterans although women comprise 9.5% of U.S veterans. Interventions that support more equitable PrEP service implementation for women is needed.

Table. Number of persons prescribed PrEP by characteristics, Lj, Veteran Health Administration data, 2017-2021

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
<th>EAPC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>3,682</td>
<td>1,284</td>
<td>2,398</td>
<td>24.0% (19.0–29.3)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>1,156</td>
<td>327</td>
<td>829</td>
<td>29.3% (24.0–34.7)</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>609</td>
<td>148</td>
<td>461</td>
<td>29.0% (23.9–34.1)</td>
</tr>
</tbody>
</table>

A PILOT RCT ASSESSING UPTAKE OF PrEP AND CONTRACEPTION IN HAIR SALONS IN SOUTH AFRICA
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Background: Half of unintended pregnancies in sub-Saharan Africa occur among women ages 15-24, who also have high HIV incidence. Women congregate regularly without partners in hair salons; these may be useful venues for family planning and HIV prevention services. Our objective was to assess the uptake of contraception and PrEP in hair salons in South Africa.

Methods: We conducted a pilot randomized trial to evaluate uptake of a stylist-initiated, nurse-supported intervention offering contraception (oral and injectable) and tenofovir-emtricitabine for PrEP in 3 salons in urban KwaZulu-Natal. Rapid HIV testing was performed at enrollment and each time PrEP was dispensed. Women could receive contraception and/or PrEP at the initial visit or opt in at a later visit during the 12-month study. We defined uptake as the proportion of eligible women who accepted salon-based PrEP and/or salon-based contraception (first-time and new users). Control salon participants were surveyed and referred to clinic for services. We assessed predictors of PrEP uptake among intervention participants using logistic regression, including self-perceived risk of HIV, partner ≥5y older, primary sex partner having other sex partners, condomless sex trended towards alignment with PrEP use without statistical significance (in women: her condomless vaginal sex: 1.75 (95% CI 0.97–3.14), his condomless vaginal sex: 1.24 (95% CI 0.87–1.75), his condomless anal sex: 1.27 (0.83–1.94)), in men: his condomless vaginal sex 1.47 (0.77–2.80), his condomless anal sex 1.28 (0.90–1.83).

Conclusion: Among young adults, PrEP use was more common when they or their partners self-reported multiple sex partners. These results are reassuring in light of the high occurrence of PrEP discontinuation among young people.
87% opted for salon-based contraception; 39% initiated PrEP during the study. Adjusting for age, uptake of salon-based PrEP was associated only with reporting any intimate partner violence (aOR 14.5, 95% CI 1.6, 100+).

**Conclusion:** Young women in urban hair salons in South Africa found receipt of family planning and HIV prevention services in a salon acceptable, with uptake of contraception (87%) higher than that of PrEP (39%). Apart from gender-based violence, risk factors including self-perceived HIV risk, age-disparate relationships and partner having other partners were not associated with PrEP uptake. Hair salons are a novel venue for reaching young women who may not perceive themselves at risk for unplanned pregnancy and HIV.

**1000 DISPARITIES IN MPXV VACCINATION UPTAKE IN SAN FRANCISCO AMONG PLWH AND PEOPLE ON PR EP**

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**Background:** In response to the 2022 global mpox outbreak, the San Francisco Department of Public Health launched a rapid mpox vaccination rollout. To identify disparities in vaccine uptake among populations disproportionally affected by mpox and to guide future vaccine campaign strategies, we assessed the proportion vaccinated among people who are either living with HIV (PLWH) in San Francisco (SF) or who are receiving HIV pre-exposure prophylaxis (PrEP) through the municipal STI clinic in SF.

**Methods:** Data were obtained from two sources: the statewide HIV case registry and the SF STI clinic database. The study population was limited to men and non-binary individuals who have sex with men (MSM) and transgender women (TGW) who were SF residents. PLWH diagnosed prior to 6/1/2022 and people receiving PrEP as of 6/1/2022 were included. The study population was matched to mpox case and vaccination data between 6/1/2022-10/15/2022 and to the STI registry. All individuals who had received at least one dose of the mpox vaccine were considered vaccinated. Chi-square and Fisher’s tests were used to compare vaccination rates by demographic and clinical characteristics and STI history. Record linkage was conducted in SQL Server Management Studio and statistical analysis was performed in SAS 9.4.

**Results:** Overall, 286/9167 (3%) of PLWH and 23/512 (5%) of people on PrEP had a diagnosis of mpox as of 10/15/2022. Of those without an mpox diagnosis, 42% of PLWH and 65% of people on PrEP at the STI clinic were vaccinated. Among PLWH, TGW (31%), Black people (34%), and people experiencing homelessness (PEH) (24%) were less likely to be vaccinated (see table). Among people on PrEP at the STI clinic, there were no significant differences in vaccination by gender identity, race/ethnicity or age. Within each cohort, those who had an STI in the past year were more likely to be vaccinated than those who had not (66% vs. 38% among PLWH and 71% vs. 58% among those on PrEP, respectively).

**Conclusion:** In SF, a considerable proportion of MSM and TGW PLWH and those on PrEP remain susceptible to mpox infection. Though uptake of mpox vaccine among people on PrEP at the STI clinic was high across racial/ethnic groups, significant disparities remain among PLWH. Efforts to integrate mpox vaccine into routine sexual health services, and to ensure equitable access and uptake, will be important for preventing future outbreaks.

| MpxV Vaccination Among PLWH, 06/01/2022-10/15/2022 | n (%); p value |
|---|---|---|
| Vaccinated | Unvaccinated | Age (median, IQR) |
| 54 (44-61) | 57 (46-65) | <0.0001 |
| Gender (n, row %) | | |
| Male | 3562 (42%) | 4924 (58%) | |
| Female | 117 (33%) | 258 (60%) | |
| Nonbinary | 13 (65%) | 7 (55%) | |
| Race (n, row %) | | <0.0001 |
| White | 1981 (41%) | 2836 (59%) | |
| Black | 398 (34%) | 606 (66%) | |
| Hispanic | 194 (44%) | 316 (50%) | |
| Asian | 276 (49%) | 285 (51%) | |
| Other/Unknown | 193 (39%) | 296 (61%) | |
| Homeless (n, row %) | | 0.0001 |
| 82 (24%) | 267 (76%) | |

**Ongoing Studies:** Beyond the observational study, we are conducting a randomized controlled trial (RCT) to assess the effectiveness of a 3-dose PrEP intervention compared to standard care for young men who have sex with men (YMSM) and transgender women (TGW) in urban and rural areas in SF. The study is powered to detect a 15% reduction in mpox incidence and will include measures of acceptability, adherence, and HIV prevention outcomes.
1002 EVALUATING THE USE OF DOSE-SPARING VACCINATION STRATEGIES FOR MPXV

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1Fred Hutchinson Cancer Research Center, Seattle, WA, USA; Infectious Economics, New York, NY, USA

Background: The 2022 monkeypox outbreak had over 80,000 cases globally with most of them in gay, bisexual, and other men who have sex with men (MSM). In response to vaccine shortages, several countries implemented dose-sparing vaccination strategies, stretching a full-dose vaccine vial in up to 5 fractional-dose vaccines. Recent studies have found mixed results regarding the effectiveness of the monkeypox vaccine, raising the question of the utility of dose-sparing strategies.

Methods: We used an age- and risk-stratified mathematical model of an urban MSM population in the United States with approximately 12% high-risk MSM to evaluate potential benefits from implementing dose-sparing vaccination strategies in which a full dose is divided into 3.5 fractional-doses. We simulated a low (34% absolute VE) and high (68% absolute VE) fractional-dose vaccine effectiveness (VE) scenarios, corresponding to retaining 40% or 80% of the effectiveness the full-dose vaccine (see Table). Population impact was evaluated over 6-month period.

Results: We found that results strongly depend on the fractional-dose vaccine effectiveness (VE) and vaccine supply (see Table). With very limited vaccine available, enough to protect with a full-dose approximately one-third of the high-risk population, dose-sparing strategies are less beneficial provided that fractional-dose preserved at least 40% of full dose effectiveness (34% absolute VE), projecting 13% (34% VE) to 70% (68% absolute VE) fewer infections than full-dose strategies. In contrast, if vaccine supply is enough to cover the majority of the high-risk population, dose-sparing strategies can be outperformed by full-dose strategies. Scenarios in which fractional-dosing was 34% efficacious result in almost three times more infections than full-dosing.

Conclusion: Our analysis suggests that when monkeypox vaccine supply is limited and fractional-dose vaccination retains moderate effectiveness, there are meaningful health benefits from providing a smaller dose to a larger number of people in the high-risk population.

Comparison of monkeypox vaccine full-dose to dose-sparing strategies for different public health scenarios

<table>
<thead>
<tr>
<th>Vaccine Strategy</th>
<th>Full-Dose</th>
<th>Dose-Sparing (Absolute VE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vaccinated</td>
<td>3,500</td>
<td>1,750</td>
</tr>
<tr>
<td>Vaccine efficacy</td>
<td>86%</td>
<td>60%</td>
</tr>
<tr>
<td>Incidence number of new MPXV infections over 6 months</td>
<td>143</td>
<td>259 (17.1%)</td>
</tr>
<tr>
<td>Cumulative number of new MPXV infections over 6 months</td>
<td>524</td>
<td>1,079 (67.6%)</td>
</tr>
<tr>
<td>Number of cases vaccinated</td>
<td>7,500</td>
<td>3,500</td>
</tr>
<tr>
<td>Vaccine efficacy</td>
<td>86%</td>
<td>60%</td>
</tr>
<tr>
<td>Peak weekly number of new MPXV infections over 6 months</td>
<td>115</td>
<td>565 (52.7%)</td>
</tr>
<tr>
<td>Cumulative number of new MPXV infections over 6 months</td>
<td>1420</td>
<td>2,304 (63.9%)</td>
</tr>
</tbody>
</table>

1003 IDENTIFYING DISPARITIES IN MPXV VACCINATION IN A SOUTHERN ACADEMIC HIV CLINIC

Edwin W. Woodhouse, Ahmad Mourad, Emily Niehaus, Hayley Cunningham, Naseem Alavian, Sofia Zavala, Patricia Kohler, Steven Pappas, Michael A. Yarri, Nwora Lance Okeke, Cameron R. Wolfe, Gary Cox, Kristen V. Dicks, Jason E. Stout
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Background: Most cases of human mpox occur in men who have sex with men, and persons with HIV (PWH). A two dose vaccine series to prevent mpox has been approved and was first deployed in the United States (US) in mid-2022. Equitable vaccine deployment may have been impeded by barriers to healthcare including structural racism and insurance coverage, preventing targeted vaccination of high risk patients. We sought to evaluate vaccination disparities by race, insurance status, and mpox risk in our Southeastern academic HIV clinic practicality.

Methods: The Duke electronic medical record interface was used to systematically identify PWH attending our clinic between 07/01/2021-11/30/2022 and mpox vaccination status. If a patient tested positive for gonorrhea or chlamydia, or had a positive rapid plasma reagin (RPR) during this period, we deemed them “high-risk” for acquiring mpox. Kruskall-Wallis test was used to compare timing of vaccination across groups. Multivariable logistic regression was performed to establish odds of vaccination among groups of interest.

Results: We identified 2,066 PLWH. 224 (10.8%) received at least one vaccine for mpox. Among the 3441 (16.7%) that were high risk for mpox only 97 (28.2%) received a vaccine for mpox. Among patients not at high risk for mpox, 127 (7.4%) were vaccinated. Of those vaccinated, 99.6% were male, 92.9% were non-Hispanic, and 54% were Black; 71.4% had private insurance, 14.7% were self-pay, 6.3% had Medicaid, and 6.7% had Medicare. In multivariable logistic regression, patients who were White had higher odds of receiving a vaccine for mpox compared to those who were Black (OR 1.46, 95%CI 1.06-2.00). Additionally, patients who had private insurance (OR 2.01, 95%CI 1.13-3.65) or were deemed high risk for mpox (OR 4.86, 95%CI 3.59-6.59) also had higher odds of vaccination. Among all patients vaccinated, race was not associated with time to vaccination, but those who had private insurance were vaccinated earlier (median 42 days earlier compared with self-pay, p< 0.0001).

Conclusion: Among PWH followed at an infectious diseases clinic in the south-eastern US, patients who were White, had private insurance, or were at high-risk for acquiring mpox had higher odds of receiving a vaccine for mpox. Efforts to reduce racial, ethnic, and socioeconomic disparities with equitable access to vaccination, particularly in the setting of rapidly evolving public health emergencies, are urgently needed.

1004 MPXV AWARENESS, RISK REDUCTION, AND VACCINE ACCEPTANCE AMONG PWH IN WASHINGTON, DC

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The DC Cohort Executive Committee
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Background: MPXV are disproportionally affected by mpox and at high risk for severe complications. The recent mpox outbreak response included increasing awareness, encouraging behavioral changes and pre- and post-exposure vaccination. We assessed knowledge and perceptions of mpox, adoption of preventive behaviors, and attitudes towards vaccination among PWH in Washington, DC.

Methods: Data from a cross-sectional mpox survey were collected between August and December 2022 from PWH enrolled in a longitudinal HIV cohort, the DC Cohort. We conducted uni- and bivariable analyses comparing participants by vaccination status (vaccinated, plan to vaccinate, no plan to vaccinate) and by HIV risk group (MSM vs. non-MSM). We conducted multinomial regression to identify factors associated with vaccine acceptance.

Results: Among 178 PWH completing the survey (median age 55; 71% male, 81% non-Hispanic Black, 37% MSM), 162 (91%) had heard of mpox. Among 159 PWH who had heard of mpox and answered vaccination questions, 21% (n=33) were vaccinated, 43% (n=69) planned to vaccinate and 36% (n=57) did not plan to vaccinate. Comparing the 3 groups, significant differences were observed by age, gender, education, income, HIV risk group, and level of worry about mpox (all p< 0.01). Viral suppression, prior COVID and influenza vaccination, access to STI services, and STI diagnoses in the last year were not associated with vaccine status. Behaviorally, a higher proportion of vaccinated participants reported limiting their number of sexual partners (p< 0.001) and using more preventive behaviors (e.g., limiting gatherings, increased condom use, avoiding skin-to-skin contact; p= 0.034) in response to mpox. A higher proportion of MSM reported limiting their number of sexual partners compared to non-MSM (33% vs. 7%, p< 0.0001) and were more likely to be vaccinated or plan to vaccinate vs non-MSM (p= 0.001). In adjusted multinomial regression models comparing vaccinated PWH and those planning to vaccinate to those not planning to vaccinate, age (p= 0.0231) and HIV risk factor/gender (p< 0.0001) were significantly associated with vaccination status with younger PWH and MSM more likely to vaccine (Figure).

Conclusion: High levels of mpox awareness were observed among this cohort of PWH in Washington, DC with more MSM employing risk reduction behaviors and vaccination as mpox prevention strategies. Ensuring that all PWH, regardless of gender, sexual orientation, or age, understand the risks of mpox may improve vaccination uptake.
1006 RESIDENTIAL SEGREGATION AS A BARRIER TO COVID-19 BOOSTER COVERAGE IN DEEP SOUTH

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Background: Coronavirus disease 2019 (COVID-19) has been a stronger hit in Deep South compared with other developed regions in the United States, and vaccination remains a top priority for all eligible individuals. However, there are limited data regarding the progress of booster coverage in the Deep South and how the coverage varies by county and age group, which is of critical importance for future vaccine planning. Racial/ethnic disparities were found in the COVID-19 vaccination, but the vast majority of evidence was generated from studies at the individual level. There is an urgent need for evidence at the population level to reveal and evaluate the booster coverage in racial/ethnic minority communities, which could identify vulnerable communities and inform future healthcare policymaking and resource allocation. We evaluated county-level COVID-19 booster coverage by age group in the Deep South and examined its relationship with residential segregation.

Methods: We conducted an ecological study at the population level by integrating COVID-19 vaccine surveillance data, residential segregation index, and county-level factors across the 418 counties of five Deep South states from December 15, 2021 to October 19, 2022. We analyzed the cumulative percentages of county-level COVID-19 booster coverage by age group (e.g., 12 to 17 years old, 18 to 64 years old, and at least 65 years old) by the end of the study period. We examined the longitudinal relationships between residential segregation, interaction of time and residential segregation, and COVID-19 booster coverage using the Poisson mixed model.

Results: As of October 19, 2022, among the 418 counties, the median percentage of booster coverage was 40% (interquartile range [IQR]: 37.8–43.0%). Compared with elders, youth and adults had lower percentages of booster uptake. There was geospatial heterogeneity in the COVID-19 booster coverage. Results of the Poisson mixed model found that as time increased, higher segregated counties had lower percentages of booster coverage. Such relationships were consistent across the age groups.

Conclusion: The progress of county-level COVID-19 booster coverage in the Deep South was slow and varied by age group. Residential segregation precluded the county-level COVID-19 booster coverage across age groups. Future efforts regarding vaccine planning should focus on youth and adults. Healthcare facilities and resources are needed in racial/ethnic minority communities.

1007 SARS-CoV-2 VACCINE EFFECTIVENESS IN A COHORT OF ACTIVE-DUTY US MILITARY PERSONNEL

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VIRAMP Study Group
1Armed Forces Research Institute of Medical Sciences in Bangkok, Bangkok, Thailand, 2Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense, McLean, VA, USA, 3Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense, Houston, TX, USA, 4Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense, McLean, VA, USA, 5Brooke Army Medical Center, Lackland, TX, USA

Background: The US Defense Dept launched its COVID-19 vaccination program in Dec 20. The VIRAMP study was designed to address knowledge gaps in US military personnel including vaccine effectiveness against asymptomatic infection, viral carriage and transmission, and durability of protection.

Methods: Military members who had received ≥1 dose of an FDA-authorized COVID-19 vaccine were enrolled at 3 sites in Texas May 21–Mar 22 and followed up for to 24 months after first dose. Study activities comprised of three in-person study visits and remote data collection: weekly and monthly questionnaires, self-collection of blood (monthly) and saliva twice weekly (more frequently if symptomatic). Participants shipped self-collected specimens for Ab analyses and SARS-CoV-2 PCR and sequencing. We report an interim analysis on data collected through May 22.

Results: Participants included 957 military members (60% male, 40% female), with 69% identifying as White, 15% Black/African American, 23% LatinX. Participants were Officers (38%) and Enlisted (62%); 54% were healthcare workers. The majority (92.5%) received the Pfizer/BioNTech monovalent/Moderna COVID-19 vaccine; 30% of participants received one booster dose. One or more breakthrough infections (btI), defined as positive saliva SARS-CoV-2 PCR, were detected in 228 (24%) participants (36 Delta, 192 Omicron). No differences were detected in rates of symptomatic vs asymptomatic btI by variant or time since last vaccine. Mean age was greater for participants with btI vs those without (35.4 (+/- 7.7) years vs 32 (+/- 8.2) years; p < 0.0001), but no differences were noted by sex, race, or ethnicity. Symptomatic infections (defined as ≥2 symptoms) were detected in 43% of participants, whereas 35%
of bti were asymptomatic; there were no hospitalizations or deaths. A trend towards reduced duration of saliva positivity was noted in Omicron infections in the 4 months following booster dose compared to infections in the 4 months following primary series (5.3 days vs 12.4 days; p = 0.0645).

Conclusion: Approximately 1/4 of participants had bti in the first year, spanning the evolving epi and vaccination landscape of the pandemic, with about 1/3 demonstrating asymptomatic infection. A trend towards shorter duration of viral carriage following booster dose was noted in Omicron infections. The VIBRAMP study demonstrated that prospective surveillance in a large, diverse cohort of US military members utilizing remote specimen and questionnaire collection is operationally feasible.

1008 EFFECTS OF PUBLIC HEALTH INTERVENTIONS AGAINST COVID-19 IN FRANCE
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1McGill University, Montreal, QC, Canada; University of Bordeaux, Bordeaux, France
Background: Non-pharmaceutical interventions (NPIs) and vaccines have been used by many countries to manage the dynamics of the COVID-19 pandemic. Despite numerous studies, considerable uncertainty remains about the effects of these public health interventions due to data quality issues and methodological challenges to estimating effects. However, producing accurate and precise estimates of the effects of these interventions is of utmost importance for the preparedness of any new epidemic.

Methods: We developed a population-based mechanistic compartmental model that includes the effect of NPIs on SARS-CoV-2 transmission and the effect of vaccination on the transmission and the rate of hospitalization. Our statistical approach estimated all parameters in one step, thus accurately propagating uncertainty, and representing spatial heterogeneity. We fitted the model to all available epidemiological data (hospital admissions and occupancy, cases, and deaths) from March 2020 to October 2021 in France. Hence, we estimated the time-varying transmission rate, and derived the effect of NPIs through an integrated regression model. We simulated counterfactual scenarios of the interplay of NPIs and vaccine availability and rollout with the same model.

Results: We found that the first lockdown reduced transmission by 84% (95% CI [81-85]) and was more effective than the second and third lockdowns (reduction of 75% [72-77] and 9% [6-13], respectively). A 6pm curfew was more effective than an 8 pm curfew (transmission reduction of 69% [67-70] vs. 50% [48-53]). School closures had a smaller effect on transmission (15% [12-19]). By the end of the study period, the protection conferred by vaccines against hospitalization and against infection, considering viral variants and population vaccine coverage, ranged between 69-92% and 29-40%, respectively. In a scenario without vaccines, we predicted 209% (95% PI [34-520]) more deaths (403 vs 403) and 346% [101-798] more hospitalizations throughout the study period. Conversely, if an effective vaccine had been available after 100 days, 65% (95% CI [83-85]) and was more effective than the second and third lockdowns (transmission reduction of 69% [67-70] vs. 50% [48-53]). School closures had a smaller effect on transmission (15% [12-19]).

Conclusion: Our results provide reliable effect and uncertainty estimates of each NPI and demonstrate that NPIs and vaccination synergistically reduced COVID-19 transmission, hospitalization, and deaths. This emphasizes the importance of stringent NPIs and a high vaccination rate to prevent further epidemic resurgences and control other emerging respiratory infectious diseases.

1009 EQUITY-BASED OPTIMIZATION FOR COVID-19 VACCINE ALLOCATION
Erin Stafford1, Dobromir Dimitrov1, Rachel Ceballos2, Laura Matrajt3
1University of Washington, Seattle, WA, USA; 2Fred Hutchinson Cancer Research Center, Seattle, WA, USA
Background: Despite the development of safe and effective vaccines and antiviral treatments against COVID-19, marginalized racial/ethnic groups in the United States continue to be disproportionally burdened by COVID-19. In response to this inequity, public health officials in several states designed, usually in an ad-hoc manner, policies aimed to be more equitable in both access and distribution of COVID-19 interventions.

Methods: We constructed an age- and race-stratified mathematical model of SARS-CoV-2 transmission and COVID-19 vaccination. We fit our model to data from Oregon at the beginning of 2021. Next, we explored counterfactual scenarios where we determined the optimal use of limited amounts of vaccine over the first 4 months of 2021 with the goal of minimizing 1) number of deaths or Years of Life Lost (YLL), 2) the inequity in mortality or YLL between race groups, 3) a combination of both. We compared them to a base-case scenario without vaccination.

Results: When vaccine supply is very limited (enough to cover 10% of the population), there is a trade-off between minimizing mortality or minimizing inequity (Fig.1). For minimizing mortality, it is optimal to allocate vaccine to the oldest age group, irrespective of race. To minimize inequity, vaccine needs to be allocated first to the marginalized populations in the young- and middle-aged groups, incurring significantly more deaths in all groups, including the marginalized ones, compared to minimizing mortality (Fig.1). When minimizing both deaths and inequity, the optimal vaccination strategy achieved a significant reduction in inequity while preserving most of the reduction in mortality (Fig.1). When minimizing YLL and inequity, the optimal allocation resulted in a more equitable distribution of resources and outcomes across race groups.

Once vaccine supply was enough to cover 20% of the population, our results suggest that it is possible to minimize both mortality (or YLL) and inequity, by protecting marginalized communities and the oldest populations at the same time.

Conclusion: With low vaccine supply, there is a trade-off between being more equitable and reducing mortality. This is true because COVID-19 related mortality is concentrated in the oldest population while marginalized populations are predominately young. This trade-off quickly disappears when more vaccine is available. An interdisciplinary approach is needed to address the inequitable distribution of resources and outcomes in public health.

1010 VACCINE UPTAKE IN A PREDOMINANTLY BLACK AND LATINX COHORT HOSPITALIZED FOR COVID-19
Michelle Chang1, Jennifer Chang1, Simon Huang2, Joan Bosco3, Meredith McNairy1, Sade Tukuru1, Yi Hao Wu1, Jonathan Kunkel-Jure1, Jessica Weidler1, Carlie Dorr1, Renee Robert3, Brett Gray1, Jason Zucker1, Delivett Castor1, Magdalena E. Sobieszczuk1
1Columbia University Medical Center, New York, NY, USA; 2Columbia University Irving Medical Center, New York, NY, USA; 3Columbia University, New York, NY, USA
Background: Vaccine uptake has been notably lower in minoritized populations in the United States. The impact of previous infection with SARS-CoV-2, disease severity, and persistent symptoms on the uptake of COVID-19 vaccines and boosters in predominantly Black and Latinx communities has not been examined. We aimed to describe correlates of vaccine uptake in a minoritized cohort hospitalized for COVID-19 during the first pandemic wave in New York City, and investigate whether those with more severe initial COVID-19 and persistent symptoms would be less likely to get vaccinated.

Methods: This retrospective cohort study included the electronic medical records of the first 894 consecutive adult patients who survived hospitalization for COVID-19 at a large quaternary care medical center in Northern Manhattan between 1 March and 8 April 2020. We abstracted data regarding demographics, comorbidities, oxygen requirements during hospitalization, persistence of symptoms at 3- and 6-months after admission, COVID-19 vaccinations through November 2022, and influenza vaccination during the 2018-2019 through 2021-2022 seasons. Unadjusted and adjusted logistic regression analyses were conducted to describe the predictors of COVID-19
vaccination, delayed vaccination (first dose after 6 May 2021), and receipt of a booster vaccine. Statistical analyses were performed using R V 4.2.1.

Results: The cohort of 894 patients was predominantly Latinx (54%) and Non-Hispanic Black (15%). 41% received at least one influenza vaccine pre-COVID, and 67% had at least one comorbidity. 22% (199/894) remained COVID-19 unvaccinated. Of the individuals who received at least one dose of COVID-19 vaccine, 57% (397/695) received at least one booster. Exactly 31% (212/695) delayed vaccination. 25% (27/106) of unvaccinated individuals reported persistent generalized symptoms compared to 18% (78/436) of vaccinated individuals. Multiple logistic regression showed that Hispanic/Latinx ethnicity, age 35–64, and concurrent influenza vaccination were associated with increased COVID-19 vaccine uptake. No association was found between vaccine uptake and disease severity or persistence of symptoms.

Conclusion: Achieving a deeper understanding of the factors driving vaccine hesitancy is critical to increasing and sustaining acceptance of COVID-19 vaccination especially in communities with historically low uptake of annual vaccines.

1011 PREVALENCE AND CORRELATES OF SARS-CoV-2 VACCINE HESITANCY AMONG US PEOPLE WITH HIV

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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, San Diego, CA, USA, 6Fenway Health, Boston, MA, USA, 7University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 8The Johns Hopkins University, Baltimore, MD, USA

Background: People with HIV (PWH) have a higher risk of COVID-19 morbidity and mortality. SARS-CoV-2 vaccination is highly effective in preventing severe COVID-19, although medical mistrust may contribute to vaccine hesitancy among PWH.

Methods: PWH from 6 sites in the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) completed the clinical assessment of patient-reported outcomes including a vaccine hesitancy instrument as part of routine care from 2/21-4/22. Participants were defined as vaccine hesitant if they had not yet received the SARS-CoV-2 vaccine and would probably or definitely not receive it. We assessed factors associated with SARS-CoV-2 vaccine hesitancy using logistic regression, and adjusted for demographics, unvaccinated viral load >200 copies/mL, calendar month and time on ART.

Results: Overall, 1,278 PWH with a median age of 55 responded; 19% were female sex at birth; 93% were virally suppressed. At the time of survey, 27% were viremic. Among 242 of whom 27% (n=242; 95% CI: 0.8–0.9). Vaccine side effects were the primary concern for women; vaccine contents for Black PWH and those who were unsuppressed; and lack of perceived COVID-19 risk for youth.

Conclusion: Vaccine hesitancy was reported by approximately 7% of a U.S. multi-site cohort of PWH, and it was more prevalent among Black PWH, women, youth, those with suppressed viral loads, and residents of the South/ Midwest. The association between virologic non-suppression and vaccine hesitancy highlights the intertwined challenge of medical mistrust for both HIV and COVID-19. Although vaccine hesitancy decreased over time, renewed efforts will be needed to address concerns of PWH about the COVID-19 vaccine, given the ongoing need for revaccination with the evolution of the pandemic.

Factors Associated with SARS-CoV-2 Vaccine Hesitancy in U.S. HIV Clinics

<table>
<thead>
<tr>
<th>Factor</th>
<th>Adjusted Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex at birth</td>
<td>1.04</td>
<td>0.81–1.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Black vs. White Race</td>
<td>1.79</td>
<td>1.28–2.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age: 50 years</td>
<td>2.79</td>
<td>1.44–5.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Region vs. Northeast (Ref)</td>
<td>2.75</td>
<td>1.84–4.11</td>
<td>0.03</td>
</tr>
<tr>
<td>South/Midwest</td>
<td>1.73</td>
<td>1.12–2.66</td>
<td>0.042</td>
</tr>
<tr>
<td>Unvaccinated Viral Load</td>
<td>1.20</td>
<td>1.14–1.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Virologic non-suppression</td>
<td>0.79</td>
<td>0.68–0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Years on ART (per year)</td>
<td>1.08</td>
<td>1.04–1.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Analyses were adjusted for age, birth sex, race/ethnicity, site, time on ART, viral suppression, and study month.

1012 SAFETY OF HETEROLOGOUS mRNA-1273 BOOST AFTER AD26.COV2.S PRIME IN SOUTH AFRICA

Nigel Garrett1, Ameena Goga1, Tarylee Reddy2, Azwidhii Takalani3, Kabushini Woebert4, Phumeza Jonas5, Imike Engelbrecht6, Ishen Seocharan7, Jacqueline Odhiambo8, Kentse Khuto9, Nonhlaniyanda Zume-Zuma9, Kate Anteyi10, Brett Leav10, Linda Gail Bekker3, Glenda E. Gray3

SHERPA Study Team

1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2South African Medical Research Council, Durban, South Africa, 3Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Johannesburg, South Africa, 4Right to Care, Johannesburg, South Africa, 5South African Medical Research Council, Cape Town, South Africa, 6Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 7Moderna, Cambridge, MA, USA, 8University of Cape Town, Cape Town, South Africa, 9South African Medical Research Council, CAPE TOWN, South Africa

Background: Given the paucity of data on safety and effectiveness of mRNA-COVID-19 booster vaccinations in lower income settings with high HIV prevalence, we evaluated a heterologous mRNA-1273 (Moderna) boost after priming with 1 or 2 doses of Ad26.COV2.S (Janssen, Johnson & Johnson) vaccine among health care workers (HCWs) in South Africa.

Methods: SHERPA is an open-label, phase 3 mRNA-1273 booster study, nested in the Sisonke Phase 3b implementation trial, that vaccinated ~50000 HCWs with 1 or 2 doses of Ad26.COV2.S from Feb and Dec 2021. Sisonke participants were offered mRNA-1273 boosters between 23 May and 12 Nov 2022 (median 17 and 8 months after 1 and 2 Ad26.COV2.S, respectively), with data cut-off on 12 Dec 2022. Reactogenicity and adverse events (AEs) were self-reported via an online data entry link shared by SMS with participants 1, 7 and 28 days after boosting. Using national databases analyses are underway to compare effectiveness against COVID-19 infections and severe disease with Sisonke participants who did not receive the booster.

Results: 12188 HCWs (79.5% female, 28.6% with self-reported previous COVID-19 diagnosis) received a mRNA-1273 booster, of whom 44.6% and 55.4% had received 1 and 2 prior Ad26.COV2.S vaccines in Sisonke, respectively. 3056 (25.2%) reported being HIV positive, more among those receiving only 1 previous Ad26.COV2.S (26.8% vs 23.9%), and 1.4% reported not being on antiretroviral therapy. 17.0% of participants reported hypertension and 6.4% diabetes mellitus. 262 participants (2.1% of women, 2.5% of men) reported 234 reactogenicity events and 95 AEs post-vaccination, with more reported by those with prior COVID-19 infection (3.5% vs 1.6%), HIV negative status (2.5% vs 1.2%) and those who received 2 prior doses of Ad26.COV2.S (2.4% vs 1.8%) (Table). Among 153 (1.3%) reporting injection site reactions the commonest were pain (59.7%), swelling (42.1%) and induration (20.1%). Of 177 (1.5%) systemic reactogenicity events (all grade 1 or 2 severity), the commonest were fatigue (59.7%), myalgia (59.7%), chills (56.8%) and headache (56.8%). 3056 (25.2%) reported being HIV positive, more among those receiving only 1 previous Ad26.COV2.S (26.8% vs 23.9%), and 1.4% reported not being on antiretroviral therapy. 17.0% of participants reported hypertension and 6.4% diabetes mellitus. 262 participants (2.1% of women, 2.5% of men) reported 234 reactogenicity events and 95 AEs post-vaccination, with more reported by those with prior COVID-19 infection (3.5% vs 1.6%), HIV negative status (2.5% vs 1.2%) and those who received 2 prior doses of Ad26.COV2.S (2.4% vs 1.8%) (Table). Among 153 (1.3%) reporting injection site reactions the commonest were pain (59.7%), swelling (42.1%) and induration (20.1%). 177 (1.5%) systemic reactogenicity events (all grade 1 or 2 severity), the commonest were fatigue (59.7%), myalgia (59.7%), chills (56.8%) and headache (56.8%). 13 COVID-19 infections occurred a median of 125 days post booster vaccination (IQR 90–154) after 3477 person-years of follow up.

Conclusion: A mRNA-1273 booster administered after 1 or 2 doses of Ad26.COV2.S was well tolerated regardless of HIV status, other chronic conditions or prior COVID-19 infection.

Multivariable logistic regression model of local/systemic reactions adjusted for age and gender.
1013 REACTOGENICITY AMONG PEOPLE LIVING WITH HIV AFTER mRNA-1273 VACCINATION IN UBUNTU STUDY
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Background: The tolerability of mRNA COVID-19 vaccines among people living with HIV (PLWH) has been understudied in vaccine trials. CoVPN 3008 (Ubuntu) is the largest multicenter Phase 3 efficacy trial of mRNA vaccines in sub-Saharan Africa.

Methods: We enrolled adults age ≥18 years living with HIV or another comorbidity associated with severe COVID-19. Previously vaccinated individuals were excluded. Baseline testing included HIV, CD4 count and HIV viral load (VL) (if HIV+), anti-SARS-CoV-2 antibodies, and nasal swab SARS-CoV-2 nucleic acid amplification test (NAAT). All participants receive vaccinations at months 0 and 6, and SARS-CoV-2 seronegative individuals also receive vaccination at month 1. This analysis includes mRNA-1273 vaccinations at months 0 and 1. Reactogenicity (solicited adverse events [AEs]) was assessed among a representative subset of participants (Safety Subset, SS) for 7 days post-vaccination. Baseline characteristics associated with moderate/severe reactogenicity events were assessed by univariate and multivariate logistic regression.

Results: 14,002 participants were enrolled in the trial (1510 into the SS) at 46 sites from 2 Dec 2021 to 9 Sep 2022. At baseline in the SS, 71% (1065) were female, median age 38 years (IQR 32-46), and median BMI 25.0 (IQR 20.7-30.2). 73% (1108) were SARS-CoV-2 seropositive, and 8.7% (131) had a positive nasal NAAT swab. 16% (197) had a history of tuberculosis. 84% (1267) were PLWH, 73% (1108) were SARS-CoV-2 seropositive, and 8.7% (131) had a positive nasal swab SARS-CoV-2 nucleic acid amplification test (NAAT) (14% (28) were PLWH, with median CD4 count of 614 cells/µL (IQR 414-861); 7% had CD4 count < 200. 21% (238) had detectable HIV VL (≥50 copies/ml), with median VL 1660 (IQR 182-2392). 14% (172/1262) and 12% (64/542) of PLWH reported moderate/severe reactogenicity after the 1st and 2nd vaccination (Figure), with no hospitalizations. Female PLWH and CD4 count <500 had 35% (p=0.03) and 44% (p=0.04) increased odds of moderate/severe reactogenicity, respectively. Other baseline characteristics were not associated with the odds of reporting moderate/severe reactogenicity among PLWH after 1st vaccination. Similar trends were seen after the 2nd vaccination, but none reached statistical significance. In multivariate models, female sex remained associated with increased odds of moderate/severe reactogenicity after the 2nd vaccination.

Conclusion: Similar to observations in HIV-negative populations, mRNA-1273 was well tolerated by PLWH with more reactogenicity in females. Impaired inflammatory responses among participants with CD4 counts < 500 cells/µL may explain less moderate/severe reactions.

Solicited adverse events after 1st and 2nd mRNA-1273 vaccination in PLWH

1014 COVID-19 VACCINATION IN PERSONS WITH AND WITHOUT HIV: VETERANS AGING COHORT STUDY
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Background: COVID-19 vaccination is effective at preventing symptomatic infection, hospitalization, and death from COVID-19, but many people have experienced barriers to receiving this life-preserving intervention. A study examining COVID-19 vaccination in New York state found that persons with HIV (PWH) were less likely to be vaccinated than the general population. We examined whether PWH are less likely to be vaccinated than persons without HIV (PW0H) in the Veterans Affairs (VA) Healthcare System.

Methods: We examined COVID-19 vaccination receipt by HIV status in the Veterans Aging Cohort Study (VACS), an open cohort of PWH and 1:2 age-, race/ethnicity-, sex-, and site-matched PW0H. Among participants with a VA encounter from 10 December 2020 to 12 September 2022, we calculated the proportion of individuals who were fully vaccinated and boosted. Fully vaccinated was defined as: 14 days after second dose of mRNA vaccine (either Pfizer BNT162B2 or Moderna mRNA-1273) or single dose of a viral vector vaccine (Janssen Ad26.COV2.S). Boosted was defined as an additional vaccination at least 180 days after full vaccination. We assessed differences using chi-square tests.

Results: Among 109,421 participants, PWH (n=31,337) were more likely than PW0H (n=78,084) to be fully vaccinated (77.6% vs 68.7%, p<0.001) and boosted (71.1% vs 63.0%, p<0.001) (Table). Most people received an mRNA vaccine with 6.9% of fully vaccinated PWH and 7.5% of fully vaccinated PW0H receiving the Janssen vaccine. Among PWH, having an undetectable HIV viral load was more common in those fully vaccinated than those not fully vaccinated (79.4% vs 72.0%, p<0.001).

Conclusion: In a matched cohort of veterans with and without HIV in VA care, we found that PWH were more likely than PW0H to be fully vaccinated and boosted. These findings contrast with a New York state study which found lower COVID-19 vaccination rates in PWH, possibly due to differential healthcare access; all patients in our cohort have access to VA care. Further studies are needed to understand differences in vaccine acceptance and receipt to prevent COVID-19 hospitalizations and deaths.

COVID-19 Vaccination in People with HIV (PWH) and People without HIV (PW0H) - Veterans Aging Cohort Study, as of 12 September 2022

1015 THE CHANGING IMPACT OF VACCINES IN THE COVID-19 PANDEMIC: A MODELING STUDY
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Background: Much of the world’s population had already been infected with COVID-19 by the time that the Omicron variant emerged at the end of 2021, but the scale of the Omicron wave was larger than any that had come before or since, and left a global imprinting of immunity which changed the COVID landscape. In this study, we explore the changing value of vaccines in a landscape of dynamic immunity and rapidly evolving variants of concern.

Methods: We use Covasim, an established agent-based model of COVID-19 enhanced with detailed intra-host dynamics. First, we simulate a vaccine trial over March 2020 — April 2022 within a population resembling that of South Africa, and estimate how both vaccine efficacy (reduction in the risk of severe disease for vaccinated vs unvaccinated individuals) and efficiency (number of doses needed to avert a death) change as the population experiences waves of wild-type, Beta, Delta, and Omicron infections. Next, we introduce six
hypothesised variants starting from February 2022 and evaluate the impact of (a) the existing set of vaccines, and (b) vaccines specifically targeted to the new variants.

**Results:** We estimate that within our simulated population, vaccine efficacy against severe disease decreased from 80% to 20% in the wake of the first wave of wild-type COVID-19, then increased back to ~70% over the latter half of 2020 as population immunity waned. This pattern repeated following each subsequent wave of infections, with vaccine efficacy falling to its lowest (10%) in the immediate wake of the Omicron wave in December 2021. The efficiency of vaccination decreases over time at an increasing rate: at peak efficacy, fewer than 100 doses would have been required to avert a single death, but by the end of January 2022, we estimate that nearly 4,000 doses would be required to avert a single death.

We find that variant-chasing vaccines will only add value above pre-existing vaccines if we can shorten the window between variant introduction and vaccine deployment to under three weeks, an impossible time-frame without significant NPI use.

**Conclusion:** Although the vaccines have proven to be remarkably effective, our work demonstrates that the population immunity acquired over the first two years of the pandemic significantly reduced the impact per dose of doses delivered after this time. Next-generation vaccines to fight future COVID variants and/or other respiratory diseases must be delivered rapidly at scale for vaccine strategies to be maximally effective.

1016 HIV VIRAL LOAD AND TIME-TO-COVID-19 VACCINATION AMONG PEOPLE WHO INJECT DRUGS

**Methods:** We included 960 adult PWUD participating in the ALIVE (AIDS Linked to the Intravenous Experience) longitudinal study in Baltimore, Maryland, who were alive and in follow up as of April 2020. We abstracted COVID-19 vaccination data from electronic medical records linked to participants via the regional health information exchange. We conducted survival analysis to estimate time from broad vaccine eligibility (April 6, 2021) to completion of the COVID-19 vaccination primary series (by HIV status (uninfected, virally suppressed PWH) and demographic and comorbidity factors) and Cox proportional hazards regression model to estimate the log-rank tests for homogeneity among strata (p-value).

**Results:** Our sample (N=960) was primarily black (77%) and male (65%) with 31% reporting recent injection drug use. Among 265 people living with HIV (PWH) with unsuppressed viral load, its critical to understand the role of HIV care among other factors associated with timely vaccination. We aimed to assess the role of HIV care on COVID-19 vaccination uptake among PWID.

**Methods:** We conducted a cohort study to evaluate coverage of the initial COVID-19 vaccine primary series and factors associated with the completion in adult PWH (≥18 years) enrolled in 8 healthcare organizations participating in the Vaccine Safety Datalink (VSD) project during December 1, 2020–December 31, 2021. Completion of two doses of the Pfizer-BioNTech or Moderna mRNA COVID-19 vaccines or one dose of the single-dose Janssen COVID-19 vaccine was assessed. Multivariable analysis was conducted using a robust Poisson regression model to estimate the rate ratio (RR) for factors associated with primary series completion, accounting for follow-up time.

**Results:** A total of 22,063 PWH were identified, among which 89% were male and 93% were viral suppressed (viral load, VL ≤200 copies/ml). Chronic comorbid conditions were prevalent, with 25% having a Charlson comorbidity score of 1-2 and 13% having a score of 3 or greater. About 23% were overweight and 17% were obese. The majority (90%) completed the primary series and 1,782 PWH (8%) did not receive any dose during the study period. A rapid uptake was achieved within the 6 months after the national COVID-19 vaccination program launched on December 14, 2020. (Figure 1) PWH who received one dose of mRNA vaccine (i.e., partially vaccinated) were excluded (n=314) from the analysis for the primary series completion. Having received an influenza vaccination in the past 2 years was the strongest predictor of completion (RR=1.17, 95%CI: 1.15, 1.20). Males (RR=1.06, 95%CI: 1.04-1.08) and those of Asian race (RR=1.05, 95%CI: 1.03-1.06, vs. White) were more likely to complete the primary series. However, PWH with baseline CD4 counts < 200 (RR=0.97, 95%CI: 0.94-0.99) and those failing to achieve viral suppression (VL>200 copies/ml) were less likely to complete the primary series. Body mass index, Charlson comorbidity score, and neighborhood household income level were not associated with completion.

**Conclusion:** Coverage of the COVID-19 vaccine primary series was high in adult PWH in the VSD. However, targeted vaccination outreach is warranted for PWH with low CD4 counts and uncontrolled HIV viral load.
1018 CORRELATES OF COVID-19 VACCINE UPTAKE IN MALAWIAN ADULTS

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Background: COVID-19 vaccine uptake has been suboptimal in many low-income countries. In Malawi, as of end-2022, just over 3.1 million adults have been fully vaccinated, representing ~21% of the adult population. We sought to identify correlates of COVID-19 vaccination among adults in Malawi to inform evidence-based policies and programs.

Methods: A survey was administered among adult (aged ≥18) clients at 32 health facilities across Malawi (May-June 2022). We asked about COVID-19 vaccination history and about hypothesized correlates per the WHO Behavioural and Social Drivers of Vaccination model: what people think and feel, social processes, and practical issues. We assessed correlations between these and vaccination status, adjusting for age, HIV status, sex, educational attainment, household wealth, and urban-rural classification using multivariable logistic regression.

Results: Surveys were conducted with 837 people, median age 39 (IQR 30–49), 56% female, 51% living with HIV and on ART. 33% were up-to-date on COVID-19 vaccination per Malawi guidelines (1 dose for J&J; 2 doses of AstraZeneca or Pfizer vaccines), 61% were unvaccinated, and 6% were overdue for a second dose, with no difference by HIV status, religion, or urban-rural classification. Up-to-date individuals were older than those who were not (45 vs 38 years, p < 0.001). The strongest correlates of up-to-date vaccination were believing the vaccine is important and safe, believing vaccination’s benefits outweigh its risks, and perceiving social support for vaccination (Table). Of 510 unvaccinated respondents, 54% had been offered the vaccine; the most commonly reported reasons for being unvaccinated were concerns about vaccine side effects (33%) and access-related barriers, such as travel time or cost (19%). Among the unvaccinated, 54% were eager or willing to be vaccinated, 29% were ambivalent, and 18% were opposed. Those opposed were less concerned about COVID-19 infection, did not feel the vaccine is important, and were less confident in the vaccine’s safety.

Conclusion: Up-to-date COVID-19 vaccination status was associated with positive attitudes about its importance and safety and perceiving pro-vaccination social norms. Concerns about vaccine side effects were common, but half of unvaccinated respondents were willing to get vaccinated. Disseminating messages about vaccine safety and ensuring local availability of the vaccine may help address concerns and access barriers, and thus help increase COVID-19 vaccination in Malawi.

Table: Correlates of up-to-date COVID-19 vaccination status

<table>
<thead>
<tr>
<th>Factor</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Believing the vaccine is important</td>
<td>3.12 (2.43–3.99)</td>
</tr>
<tr>
<td>Believing the vaccine is very safe</td>
<td>2.98 (2.29–3.87)</td>
</tr>
<tr>
<td>Social support</td>
<td>1.54 (1.19–1.98)</td>
</tr>
<tr>
<td>Being male</td>
<td>0.65 (0.50–0.85)</td>
</tr>
<tr>
<td>Tertiary education</td>
<td>1.09 (0.88–1.35)</td>
</tr>
<tr>
<td>Living with HIV</td>
<td>1.09 (0.88–1.35)</td>
</tr>
<tr>
<td>Urban residence</td>
<td>1.03 (0.88–1.23)</td>
</tr>
<tr>
<td>Having been offered the vaccine</td>
<td>2.27 (1.75–2.94)</td>
</tr>
</tbody>
</table>

Conclusion: Correlates of up-to-date COVID-19 vaccination status

1019 SAFETY OF Ad26.COV2.S AND AZD1222 COVID-19 VACCINES AMONG ADULTS IN MALAWI

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Background: The safety profiles of the Ad26.COV2.S and AZD1222 COVID-19 vaccines have not been described in a general population in Malawi. We present self-reported adverse reactions (AE) following receipt of these vaccines in Malawi as part of a phone-based syndromic surveillance survey.

Methods: We conducted phone-based syndromic surveillance surveys among adults (≥18 years) with verbal consent from July to April 2022. We used secure tablets through random digit dialing to randomly select mobile phone numbers and electronic data collection forms. Survey questions included whether the respondent had received at least one dose of the COVID-19 vaccines, whether they had experienced any AE following vaccination, and the severity of the AE. We used multivariable analysis to identify factors associated with self-reported adverse reactions post-COVID-19 vaccination.

Results: A total of 11,924 (36.0%) out of 33,150 participants reported receiving at least one dose of either Ad26.COV2.S or AZD1222 between July–December 2021; 65.1% were female. An estimated 49.2% of the vaccine recipients reported at least one AE, 90.6% of which were mild, and 2.6% were severe. About 16.9% (n = 656) of respondents who received the first dose of AZD1222 had AE, while 50.2% (n = 2,823) of those who received the second dose of AZD1222 and nearly all individuals (n = 2,385) who received Ad26.COV2.S reported AE.

Joint pain (45.5%), fever (26.7%), headache (26.1%), pain at the injection site (24.4%), and fatigue (16.6%) were among the commonly reported AE. Males were less likely to report an AE compared to females (Adjusted Odds Ratio (AOR) 0.81 95% confidence interval (CI) 0.75–0.88). Older age was associated with reduced odds of an AE compared to those aged 18–24 years: 65+ years: (AOR 0.62, 95% CI 0.50–0.77). The likelihood of reporting AE increased with education level: tertiary education AOR 2.63 95% CI 1.96–3.53. Respondents who thought COVID-19 vaccines were not safe were more likely to report post-vaccination adverse reactions than those who thought it was very safe (AOR 1.44, 95% CI 1.30–1.61).

Conclusion: Ad26.COV2.S and AZD1222 vaccines are well-tolerated, with primarily mild and few severe AE among adults living in Malawi. Self-report of AE following COVID-19 vaccine receipt is associated with gender, age, education, and concern about the safety of the vaccines. Recognizing these associations is key when designing and implementing COVID-19 vaccination communication messages to increase vaccination coverage.

1020 COMPARISON OF THE EFFECTIVENESS OF DIFFERENT COVID-19 VACCINES AMONG PLWHL

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Background: Previous studies have demonstrated promising serologic responses in PLWH receiving a third dose of COVID-19 vaccine. However, real-world clinical effectiveness, especially during the pandemic caused by B.1.1.529 variant, remains less investigated.

Methods: PLWH seeking HIV care at our hospital from 2021/6 to 2022/6 were included and advised to receive the third dose of COVID-19 vaccine. Individuals were excluded from this study if they had been previously diagnosed with COVID-19. Different types of COVID-19 vaccines were available in the vaccination program, including BNT162b2, mRNA-1273 (either 50 or 100 μg), MCV COV1901 and NVX-CoV2373 vaccines. PLWH were screening for the occurrence of COVID-19 through the reporting system of notifiable diseases of Taiwan CDC, and were tested for anti-nucleocapsid (anti-N) IgG every 1 to 3 months.

Results: 1,496 PLWH were included: 631 (42.2%) receiving 100 μg mRNA-1273 vaccine, 468 (31.3%) 50 μg mRNA-1273 vaccine, and 328 (21.9%) BNT162b2 vaccine, 468 (31.3%) 50 μg mRNA-1273 vaccine, and 328 (21.9%) BNT162b2 vaccine, 1,496 PLWH were included: 631 (42.2%) receiving 100 μg mRNA-1273. 297 (19.9%) PLWH were diagnosed with COVID-19 during the follow-up period, including 92 (14.6%) who received
1021 COMPARISON BETWEEN HIV+ WOMEN TRANSITIONING THROUGH MENOPAUSE AND MEN OF SIMILAR AGE

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Swiss HIV Cohort Study (SHCS)
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Background: Not much is known on differences in treatment - adherence, psychiatric health and HIV-viral control between women transitioning through menopause and men of similar age, risk group and treatment history. With this study, we aimed at closing this knowledge gap.

Methods: We identified 1,437 HIV-positive women with menopause onset between 01/2010 and 12/2021, and 1,094 men of similar age from the same time period in the Swiss HIV Cohort Study (SHCS). We considered data on HIV-viral load, depression and/or being in psychiatric care and self-reported treatment adherence between the ages of 41 and 60. Non-adherence was defined as any person self-reporting missing one dose once every 2 weeks, or in psychiatric care, and 2.3% of women (men 2.3%) self-reported treatment non-adherence. We fitted log-binomial interrupted time series (ITS) regression models to determine factors influencing treatment adherence and compared outcomes between men and women. We adjusted for differences in risk group (heterosexual, injection drug users (IDU)), time on ART and year of visit between men and women using inverse probability weighting (IPW), and calculated sandwich-type standard errors as patients have many clinical visits.

Results: 2,531 people living with HIV attending 90,310 clinical visits were included, of which detectable HIV-RNA ( >50 copies/ml) was observed in 9.4% of women and 10.2% of men. 10.1% of women (men 8.6%) were depressed and/or in psychiatric care, and 2.3% of women (men 2.3%) self-reported treatment non-adherence.

Women had fewer visits with detectable HIV-RNA compared to men, but there was little overall difference between the ages of 45 and 52 (p=0.7, Figure A1). Women were slightly more likely to be depressed and/or in psychiatric care (IPW adjusted incidence rate ratio for women 1.26 (reference: men), 95% confidence interval [1.12, 1.41], p= 0.001, Figure B), but there was no difference in terms of treatment adherence (p=0.9, Figure C). Women IDU exhibited a slight increase in detectable HIV-RNA during peri-menopause (Figure A2).

Conclusion: There were no differences between men and women in terms of both detectable HIV-RNA and treatment adherence, but a slightly higher rate of being depressed and/or in psychiatric care for women of 48-53 years. Apart from women with IDU as the probable mode if HIV acquisition, there were no significant increases in viral events during peri-menopause.

Figure: Percentage of visits with detectable HIV-RNA (A), psychiatric treatment and/or depression (B) and treatment non-adherence (C) for women (blue) and men (red); age at menopause histogram on the x-axis.
increased in TW-H (p=0.001) but not CW-H. Longitudinal measurements from 27 TW confirmed our finding as a significant change of slope in CD4+ T cell dynamics occurred after the start of sex hormone intake (p<0.001). Proteomics analysis revealed activation of innate immunity pathways (MCP-2, MCP-4, CCL3, CCL4) in CW-H but not TW-H.

Conclusion: Sex hormone intake is associated with distinct modulations of the immune system in CW and TW, which could potentially be due to differences in dosing, combination of hormones taken, and/or mechanisms of actions of hormones on the immune system in CW and TW. Forest plot showing factors (parameters and 95% confidence intervals) associated with CD4 counts.

1024 THE EFFECT OF FP EDUCATION ON KNOWLEDGE AND USE OF FP IN UGANDAN FISHING COMMUNITIES

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Background: Family planning knowledge is poor and use is low in Ugandan fishing communities. We compared the effectiveness of enhanced family planning (FP) education with routine counseling on FP knowledge and use.

Methods: Individuals aged 15–49 years were randomly assigned to intervention or control arm. The intervention constituted enhanced FP education based on a simplified handout extracted from the WHO FP guidance tool called, “Family planning: A global handbook for FP providers” which participants took home for additional reading. The control arm constituted FP counseling following Uganda Ministry of Health guidelines. FP knowledge score and contraceptive prevalence rate (CPR) were compared between trial arms at baseline and at 12 months. Negative binomial regression models were used to estimate the effect of the intervention on FP knowledge and use.

Results: Overall, 1,410 participants were screened to enroll 1,004 (502 per study arm, 48.5% women). Subsequently, 384 (76.5%) and 383 (76.3%) completed the 12 months’ follow-up in the intervention and control arms respectively. At baseline, a median FP knowledge score of 8 and a < 70% FP knowledge score was observed for all participants with a CPR of 36.8%. At month 12, the median FP knowledge score improved in both arms, higher in the intervention arm than the control arm (46 vs 30; p< 0.001). In the intervention arm, 304 (79.2%) had a score of ≥70 compared with 21 (5.5%) in the control arm (p<0.001). In the negative binomial regression model, the change in FP knowledge score was 47% higher in the intervention arm than in the control arm (score ratio: 1.47, 95%CI: 1.43-1.51, p< 0.001). The change in CPR was 16% higher in the intervention arm than in control arm (Prevalence ratio: 1.16, 95%CI: 1.01-1.34, p< 0.040).

Conclusion: Enhanced FP education using a simplified FP education handout was more effective in increasing FP knowledge and use compared to routine FP counseling for people living in fishing communities. Innovative FP education interventions are recommended for improving FP knowledge and optimizing uptake in remote-rural settings where literacy levels are low.

1025 INCREASED HIV, HCV & STIS AMONG ADULTS REPORTING SEX WORK IN ALABAMA: 2008-2022

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Background: The U.S. South represents 38% of the U.S. population, but as of 2020 more than 50% of new U.S. HIV diagnoses occurred in the region. HIV and other health-related outcomes are poorer in states considered the “Deep South,” including Alabama. Relative to other states, Alabama has higher rates of HIV and other STIs, and 17% of people with HIV in Alabama are unaware of their status. Throughout the Deep South, HIV and STI epidemics are in part driven by...
socioeconomic vulnerability, and sex work may play a role. However, few studies have estimated the prevalence or effects of sex work in Alabama or the Deep South.

Methods: We estimated the history of sex work (exchanging sex for money, drugs, or something of need in the last 5 years) among clients (ages 15–64) receiving services at a Birmingham, Alabama-based AIDS Service Organization from 2008–2022. We used chi-square tests to assess sex work’s association with diverse drug use (injection and non-injection) and sexual risk behavior. We used logistic regression to examine the association between sex work and new HIV, hepatitis C (HCV), and STI (chlamydia, gonorrhea, syphilis, trichomoniasis) diagnoses. Analyses adjusted for age, race/ethnicity, and gender identity, with stratification to examine differences among gay, bisexual and other men who have sex with men (MSM, n=4,784).

Results: Across 20,673 visits, history of sex work was reported at 950 visits (4.6%). Sex work was associated with older age (p=0.002), being a cisgender woman or gender minority, identifying as non-Hispanic white, increased diverse drug use, and increased sharing of non-injection drug equipment (p<0.001). Compared to other clients, those reporting sex work had increased odds of syphilis (aOR 3.42, p=0.05) and HCV diagnosis (aOR 1.76, p<0.001). MSM with history of sex work had increased odds of diagnosis with HCV (aOR 4.57, p<0.005) and HIV (aOR 2.49, p=0.02), compared to other MSM.

Conclusion: Using 14 years of community-based data, this study is among the first to estimate the relationship between sex work and HIV, HCV, and STIs in the Deep South. We document converging sex work and diverse drug-related risks, which may act synergistically to influence poorer health outcomes and reinforce existing health inequities. Differences in HIV and HCV diagnosis between sex workers and other clients were even greater among MSM, suggesting a need for tailored interventions with this group.


Table: Logistic regression of new HIV, HCV, and STI diagnosis on sex work history (adjusting for age, race/ethnicity, and gender identity) across 20,673 visits, 2008–2022.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>1.76 (1.33–2.32)</td>
<td>&lt;0.001</td>
<td>1.15 (0.96–1.35)</td>
<td>0.25</td>
</tr>
<tr>
<td>Chlamydia (CT)</td>
<td>0.93 (0.51–1.70)</td>
<td>0.79</td>
<td>0.79 (0.41–1.50)</td>
<td>0.61</td>
</tr>
<tr>
<td>Gonorrhea (GC)</td>
<td>0.99 (0.72–1.39)</td>
<td>0.95</td>
<td>0.60 (0.27–1.35)</td>
<td>0.34</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>0.79 (0.42–1.50)</td>
<td>0.21</td>
<td>0.55 (0.42–1.30)</td>
<td>0.93</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0.42 (0.10–1.56)</td>
<td>0.03</td>
<td>0.29 (0.21–0.40)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1026 PREVALENCE OF CHLAMYDIA AND GONORRHEA AMONG ADOLESCENTS IN KISUMU, KENYA
Hong-Ma T. Truong 1, Elsa Heylen 2, Kevin Kadero 3, Sayo Amboka 4, Damaris Odeny 5, Maurice Opiyo 6, Fidel Opondo 7, Beatrice Otieno 8, Hanningtone Odhiambo 1, David Ogolla 2, Mary Gyel 9, Lara E. Miller 9, Craig R. Cohen 1, Elizabeth A. Bukusi 1
Maneno Yetu Study Team
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Background: The national diagnostic algorithm for sexually transmitted infections (STIs) in Kenya is based on syndromic management. This approach, however, underestimates STI prevalence as asymptomatic cases go undiagnosed and untreated. We conducted screening for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) for adolescents participating in a sexual and reproductive health study in Kenya.

Methods: The Maneno Yetu study recruited adolescents aged 15 to 19 residing in the informal settlements of Kisumu, Kenya. Urine screening for CT and NG was offered to 1238 participants who reported having ever engaged in sex, of whom 1167 accepted and 1159 had interpretable results. Urine specimens were screened using the molecular Xpert CT/NG test. Associations were assessed by Chi-square tests.

Results: Of the 1159 adolescents, 53% were girls and 74% were 18 to 19 years of age. Overall, 55% of adolescents were worried about acquiring an STI, 24% had ever experienced STI symptoms and 3% had a prior STI diagnosis. Girls were more likely than boys to report experiencing symptoms (29% vs. 18%; p=0.001). STI prevalence was 9.6% overall and was higher among girls than boys (12.5% vs. 6.3%; p<0.001). Of the 117 adolescents (76 girls and 35 boys) who tested positive, 102 had CT, 15 had NG, 6 had CT/NG co-infection, and 66 did not report experiencing symptoms. Ninety-two adolescents (83%) were successfully linked to care and received STI treatment. Among our sample, 33% of adolescents had engaged in transactional sex and 34% had experienced forced sex. STI prevalence was higher among adolescents who had engaged in transactional sex (13.0% vs. 7.9%; OR=1.74, p=0.006) and those who had experienced forced sex (12.2% vs. 8.2%; OR=1.56; p=0.028) compared to adolescents who did not.

Conclusion: Two-thirds of adolescents diagnosed with an STI in our study did not report any previous symptoms, and thus would most likely have gone undiagnosed and untreated based on the syndromic management diagnostic algorithm. Undiagnosed or misdiagnosed STIs can result in onward transmission and significantly impact the reproductive health of adolescents, including pelvic inflammatory disease and infertility in females. In addition, STIs are associated with an increased risk of HIV infection. Our findings lend support for offering STI screening to adolescents who report having engaged in transactional sex or experienced forced sex to enhance sexual and reproductive health and HIV prevention services.

1027 PATTERNS OF STI AMONG TRANSGENDER WOMEN LIVING WITH AND WITHOUT HIV IN THE US
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American Cohort To Study HIV Acquisition Among Transgender Women (LITE) Study Group
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Background: Transgender women (TW) bear a disproportionate burden of HIV and other STIs. There are limited data comparing the epidemiology of STIs among TW with HIV and TW without HIV. We describe the prevalence and patterns of STIs among TW in 6 eastern and southern US cities by HIV status.

Methods: We analyzed baseline data collected between March 2018 and August 2020 among adult TW across 6 US cities screened for the LITE study (N=1,018). Participants completed a sociobehavioral survey, oral HIV screening, and self-collected urine, anal, and neovaginal specimens for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) testing, and provided sera for treponemal syphilis testing and rapid plasma reagin (RPR) testing with quantitative RPR titers. Sera were assayed for herpes simplex virus type 2 (HSV-2). The primary outcome was ≥1 bacterial STI (BSTI). We examined the association of HIV status with BSTI and conducted HIV-stratified analyses to identify correlates of BSTI. For each covariate of interest, we estimated adjusted prevalence ratios (aPR) via separate modified Poisson regression models adjusted for age, race, and geographic region.

Results: Compared to TW without HIV (n=742), TW with HIV (n=276) had a higher prevalence of ≥1 BSTI (11% vs. 33%; aPR=1.96 [95%CI=1.43-2.70]). BSTI prevalence included 4% CT, 2% GC, and 6% syphilis among TW without HIV, and 8% CT, 4% GC, and 28% syphilis among TW with HIV. Compared to TW without HIV, TW with HIV also had a higher prevalence of HSV-2 antibodies (30% vs. 82%; aPR=1.53 [95%CI=1.33-1.75]). Among TW without HIV, a higher prevalence of ≥1 BSTI was associated with living in the Upper South Atlantic region (vs. Northeast), identifying as Black and/or Latinx (vs. White), identifying as gender nonbinary/genderqueer (vs. trans woman), reporting ≥1 sex partner, hazardous alcohol use, and having safety concerns regarding transit to healthcare (Figure). Receipt of gender-affirming medical services (e.g., psychotherapy, surgery) was associated with lower BSTI prevalence. Among TW with HIV, older age, hazardous alcohol use, and a lifetime history of sexual violence was associated with lower BSTI prevalence.

Conclusion: TW had a high prevalence of bacterial and viral STIs. STI prevalence was significantly higher in TW with HIV, and the correlates of BSTI prevalence differed by HIV status. Overriding the drivers of STIs unique to TW with and
without HIV will be key to informing tailored interventions and reducing sexual health-related inequities among TW.

Figure. Correlates of Bacterial STI prevalence stratified by HIV status.

**1028 SEXUALLY TRANSMITTED INFECTIONS AMONG YOUNG ADULTS AFFECTED BY PRENATAL HIV**

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Pediatric HIV AIDS Cohort Study Network

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**Background:** As young people living with perinatally-acquired HIV (YPHIV) become sexually active, they become susceptible to sexually transmitted infections (STI). Because of their HIV status, YPHIV may more frequently receive advice to use condoms consistently to protect their partner(s) from HIV, hence may be at lower risk of STIs than those HIV uninfected. The objective of this study was to compare STI incidence rates between YPHIV and a comparator group of young adults living with perinatal HIV exposure but uninfected (YPHUEU).

**Methods:** YPHIV and YPHUEU participating in the U.S.-based Pediatric HIV/AIDS Cohort Study (PHACS) network had test results for chlamydia (CT), gonorrhea (GC) and trichomonas (TV) abstracted annually from medical charts. Additionally, participants were tested annually by Hologic Aptima® assay for CT, GC, TC. Women were also tested for human papillomavirus (HPV) via Aptima® assay. These research samples were sent for testing in batches to a centralized laboratory. Incidence rates of first positive test in AMP Up were calculated for CT, GC, and TC over person-time of follow-up as of January 1, 2020. HPV incidence rates were calculated among those with an initial negative HPV test result. Poisson regression with robust standard errors was used to calculate incidence rate ratios (IRR) and 95% confidence intervals (95% CI) comparing rates by HIV status, age at first STI test, sex at birth, race, and ethnicity.

**Results:** Among 579 AMP Up participants (83% PHIV; 62% female) YPHIV were older than YPHUEU at first STI test (mean age 22.9 vs 19.1 years). Incidence rates of CT, GC, and TV were similar by HIV status and age. However, incidence of HPV was higher among women with PHIV compared to those PHEU. Rates of most STIs were higher for women than men, for those of Black vs White/others) race, and non-Hispanic vs Hispanic participants (Table).

**Conclusion:** Rates of CT, GC and TV were similar between YPHIV and YPHUEU, underscoring the need for better STI prevention strategies among YPHIV, including promotion of condom use. The higher rate of HPV in women with PHIV than PHEU is consistent with the well-documented vulnerability of those with HIV for HPV and underscores the need for HPV-associated cancer surveillance. Confirmation of HPV vaccine status will be a critical next step to understanding HPV vaccine effectiveness in this group.

**Incidence** (per 1000 person-years) and **Incidence Rate Ratios** (95% confidence intervals) of first positive STI test result during AMP Up Follow-up by demographic characteristics

<table>
<thead>
<tr>
<th><strong>Outcome</strong></th>
<th><strong>TGW without HIV</strong></th>
<th><strong>TGW with HIV</strong></th>
<th><strong>Incidence Rate Ratio (95% CI)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>0.97 (0.96-0.98)</td>
<td>0.99 (0.98-1.00)</td>
<td>1.02 (0.99-1.04)</td>
</tr>
<tr>
<td>Race (ref. non-Hispanic/Latinx)</td>
<td>1.00 (0.99-1.01)</td>
<td>1.00 (0.99-1.01)</td>
<td>1.00 (0.99-1.01)</td>
</tr>
<tr>
<td>Gender (ref. male)</td>
<td>0.98 (0.97-0.99)</td>
<td>0.97 (0.96-0.98)</td>
<td>1.00 (0.99-1.01)</td>
</tr>
<tr>
<td>Geographic region (ref. Northeast)</td>
<td>0.99 (0.98-1.00)</td>
<td>0.99 (0.98-1.00)</td>
<td>1.00 (0.99-1.01)</td>
</tr>
<tr>
<td><strong>Intervals</strong> (of first positive STI test result during AMP Up)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>2.08 (1.64-2.63)</td>
<td>2.71 (2.09-3.51)</td>
<td>1.30 (1.02-1.66)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>1.75 (1.36-2.25)</td>
<td>1.63 (1.25-2.12)</td>
<td>0.94 (0.71-1.24)</td>
</tr>
<tr>
<td><strong>Conclusion:</strong></td>
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</tbody>
</table>

1029 **INCIDENCE INCREASED OF ANAL HPV-RELATED POTENTIALLY PRECANCEROUS LESIONS IN HIV+ WOMEN**

Eugenio Nelsen Cavallari, Letizia Santinelli, Motta Eleonora, Federica Tanoi, Luca Maddaloni, Ilaria Cuccu, Giancarlo Ceccarelli, Carolina Scagnolari, Alessandra Pierangeli, Innozencia Palaia, Claudio M. Mastroianni, Gabriella D’Ettorre

Sapienza University of Rome, Rome, Italy

**Background:** Women living with HIV (WLH) show increased risk of HPV related anal cancer. WLH are routinely screened to prevent HPV related cervical cancer, through research and treatment of potentially precancerous lesions, but screening for the prevention of HPV related anal cancer lacks in this population. In the present study we aimed to compare prevalence of anal HPV infection and anal dysplasia in WLH and women without HIV infection (WWH).

**Methods:** Since genital HSIL is a risk factor for anal dysplasia, women with previous or current cervical, vaginal or vulvar HSIL were excluded. 74 WWH and 26 WLH were enrolled in the present study. All participants underwent anal HPV DNA test, anal cytology and high resolution anoscopy (HRA). Data regarding genital HPV DNA and genital dysplasia (cystology and/or histology) were also collected. None of participants completed HPV vaccination course prior to enrollment.

**Results:** Median age of participants was 45 years in both groups. WWH showed lower prevalence of genital high-risk HPV (HR-HPV) (33% vs. 78%; p=0.01) and similar prevalence of low-risk HPV (LR-HPV) (40% vs. 22%; p=0.67) compared to WWH. 27% of WLH showed negative genital HPV. Screening for cervical dysplasia showed LSIL in 40% WLH and 35% WWH. Absence of dysplasia was observed in 60% WLH and 65% WWH.

Prevalence of anal HR-HPV was higher among WLH (34% vs. 14%; p=0.049), on the other hand prevalence of LR-HPV was similar between WLH and WWH (26% vs. 45%; p=0.1). Similar proportion of WLH and WWH showed negative anal HPV DNA (40% vs. 41%; p=0.8).

Cytological LSIL was observed in 40% WLH and 12% WWH (p=0.017), normal anal cytology was found in 54% WLH and 86% WWH (p=0.001).

All participants undergone HRA, biopsies were performed according to clinical evidences (33% WLH and 51% WWH). Biopsy proven HSIL was more frequently observed in WLH than WWH (20% vs. 4%; p=0.01). Prevalence of LSIL was similar between the two groups (33% vs. 22%; p=0.23).

HIV infection was associated with increased risk for histology proven anal HSIL (odds ratio 5.6; 95% CI 1.2-25.6). Receptive anal intercourse and smoking were not associated to increased risk of HSIL.

**Conclusion:** WLH show increased burden of HPV related anal dysplasia and potentially precancerous anal lesions in respect to WWH. In WLH, despite similar prevalence of anal and genital HR-HPV, anal canal seems more prone to develop severe dysplasia. WLH should be considered as a high-risk population and therefore should undergo screening for the prevention of anal cancer.

1030 **EFFICACY OF LATE HPV VACCINATION IN YOUNG HIV+ MSM**

Eugenio Nelsen Cavallari, Letizia Santinelli, Motta Eleonora, Federica Tanoi, Luca Maddaloni, Ilaria Cuccu, Giancarlo Ceccarelli, Carolina Scagnolari, Alessandra Pierangeli, Innozencia Palaia, Claudio M. Mastroianni, Gabriella D’Ettorre

Sapienza University of Rome, Rome, Italy

**Background:** Men who have sex with men (MSM), and in particular HIV+ MSM, show the greatest risk of anal HPV infection and the highest incidence of anal cancer. HPV vaccine should be ideally administered at an early age, before the first sexual intercourse. Since routine administration of HPV vaccine to young boys has begun only in recent years, currently the majority of immunized adult MSM individuals underwent vaccination after sexual debut. In the present study we aimed to evaluate vaccination rate and prevalence of anal HPV infection and
anal dysplasia (squamous intraepithelial lesion, SIL) in HIV+ and HIV- MSM aged < 45 years that received HPV vaccine after the first sexual intercourse. Methods: 142 MSM, 110 HIV+ and 32 HIV-, younger than 45 years were included in the present study. All enrolled subjects underwent anal HPV DNA test for HPV identification and genotyping. The prevalence of anal dysplasia was assessed trough anal cytology or anal histology from anal biopsies collected during high resolution anoscopy. Results: Vaccination rate was similar between HIV+ and HIV- participants (20% vs. 31.3%; p=0.169). 76.3% of HIV+ participants and 57.1% of HIV- participants tested positive at anal HPV DNA test (p=0.042). Anal SIL of any grade was observed in 76.3% of HIV+ individuals and 53.6% of HIV- subjects (p=0.017). The prevalence of anal HPV infection was similar between vaccinated and unvaccinated HIV+ subjects (72.7% vs. 77.3%; p=0.864). Among vaccinated participants, HPV DNA tested positive in 72.7% of HIV+ and 33.3% of HIV- subjects (p=0.041). On the other hand, 77.3% of HIV+ unvaccinated and 68.4% of HIV- unvaccinated individuals showed a positive HPV DNA test (p=0.415). Among HIV+ participants, anal SIL was observed in 54.4% of vaccinated and 81.8% of unvaccinated individuals (p=0.01). Among vaccinated participants, the prevalence of anal SIL was detected in 54.5% of HIV+ and 33.3% of HIV- vaccinated participants (p=0.283). In unvaccinated participants SIL was detected in 81.8% of HIV+ and 63.1% of HIV- subjects (p=0.073). Being unvaccinated (OR 1.6; CI 95% 1.1-2.4), living with HIV (OR 2.8; CI 95% 1.2-6.6) and anal HPV infection (OR 6.7; CI 95% 2.9-15.4) were associated to an increased risk of anal dysplasia at multivariate analysis. Conclusion: Even if administered after sexual debut, vaccination against HPV is useful in reducing the risk of anal dysplasia in HIV+ MSM aged < 45 years. Immunization against HPV should be encouraged, particularly in this population.

1031 PREVALENCE OF HPV, OTHER STI, AND ANAL LESIONS AMONG MSM IN TOGO

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Background: High prevalence of STI is a critical issue in Africa, especially in key populations such as MSM. Here, we present the baseline results of a 2-year longitudinal cohort study (ANRS DEPIST-H 12400) enrolling both HIV-positive and negative MSM.

Methods: MSM were included in Lomé (Togo) between June and december 2021, half of them living with HIV. High risk HPV (hrHPV) (Seegene) and HSV-1/2 (Altona) detection was performed on anal smears. Syphilis and HBS antigen were tested on sera samples on site. Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) were tested (Cepheid) from urine, pharyngeal and anal swabs. A clinical genital examination was carried out by trained physicians.

Results: 200 MSM with a median age of 23 years (IQR=21-29) were enrolled. Prevalence of each STI is shown in Table 1. Only 1.5% of participants were positive for HBS antigen, while no HCV nor syphilis infection was detected. The prevalence of CT and NG was 6.5% and 3.0% in urine, 26.0% and 22.0% in anal swab, and 5.0% and 19.0% in oropharyngeal area, respectively. Anal herpes simplex virus (HSV) infection were detected in 9 (4.5%) of MSM (1 HSV-1 and 8 HSV-2). Overall, a high prevalence of anal hrHPV was detected (75.9%) and was significantly higher among HIV-positive MSM (84.0% vs. 67.7%, p=0.008). The prevalence of hrHPV16, 18, 33, 35, 39, 51, 52, 58, 59, 66 types was 36.6% (p=0.017). Anal lesions were detected at examination in 43.0% of MSM, with 19.5% of condylomas, 17.5% of maricas, 3.0% of anal fissures while anal ulcerations, gluteal abscess, hemorrhoid related pathology and anal fistula were diagnosed in 2.0% or less of participants. No anal cancer has been diagnosed.

Conclusion: These data confirm the high burden of STIs among the key population of MSM in Togo. It also confirms the unusual distribution of HPV types in western Africa, with HPV33 being a highly prevalent hrHPV type non-covered by the nonavalent vaccine. A national strategy regarding STI screening and HPV vaccination in this key population is needed.

1032 METHYLATION MARKERS ON ANAL SMEARS ARE ASSOCIATED WITH HIGHER ANAL CANCER RISK

Valentine M. Ferré1, Axelle Dupont1, Mélanie Draullette1, Emy Valette1, Ghislain Staumont1, Morgot Bucal1, Anas Najj1, Laurent Siproudhis1, Bart Hesselink2, Dominique Bouchard3, Lucas Spindler4, Reneske D. M. Steinbergen5, Diane Descamps1, Charlotte Charpentier1, Laurent Abramowitz2, Carine Roy3, COAINS national cohort

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Background: Currently, there is no consensus about anal cancer screening. Molecular markers stratifying anal cancer risk are needed. Host cell DNA methylation markers (ZNF582 and ASCL1) have been associated to AIN3 and anal carcinoma in a cross-sectional study on biopsies from HIV-infected men.

Methods: This is an ancillary prospective study of the French COAINS cohort, which included patients with an AIN3 history. Cytology, high-risk HPV (hrHPV), p16/ki67 and methylation markers ZNF582 and ASCL1 on anal smears were compared with Wilcoxon tests. Association between anal cancer risk and methylation was evaluated in univariate Cox models. C/D time dependent AUC threshold was related to cancer risk, in univariate analysis (HR = 1.34 [1.15-1.56], p< 0.001 for both markers). The cutoff was defined to assess discrimination of both markers. Similarly to Youden Index, methylation thresholds were determined for a one year follow-up. Methylation levels were expressed in log2 (ΔΔddct).

Results: Methylation analyses were successful for 424 patients with 60% of male, 59 years-old of median age and 45% of HIV-positive patients overall, 91% of them being men. Median follow-up was 36 months [32-40] in this sub-study. Twenty out of 424 patients evolved through anal cancer in the study period. A higher methylation rate of each gene was significantly associated with HSIL cytology, HPV16 or hrHPV detection and p16/ki67 positivity (p< 0.01 for all) on anal smears at baseline. On our dataset, thresholds of 0.62 and 1.95 could be defined for ZNF582 and ASCL1 respectively, with corresponding sensitivities of 86% and 78% and specificities of 63% and 67%. Regarding cancer risk, ZNF582 methylation >0.62 had a HR=5.02 [1.67-15.1] and ASCL1 methylation >1.95 a HR=5.02 [1.67-15.1]. At least one methylation marker above the corresponding threshold was associated with higher risk of cancer in univariate analysis with a HR 4.19 [1.39-12.6]. p=0.011.

Conclusion: To our knowledge, this is the first study evaluating the potential role of methylation markers for anal cancer risk stratification in a real-life cohort on non-invasive sample such as anal smears. Further study are needed to confirm the prognostic value of these markers, notably in a less “at risk” population, with longitudinal follow-up of methylation, to define methylation thresholds that can be generalized.
**1033** MPXV AND SEXUALLY TRANSMITTED INFECTION AMONG MEN WHO HAVE SEX WITH MEN

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*Denver Health and Hospital Authority, Denver, CO, USA; Denver Health Medical Center, Denver, CO, USA*

**Background:** The majority of mpox cases in the United States have occurred among males, with a sizeable proportion reporting male-to-male sexual contact. In an effort to inform mpox testing and vaccination efforts, this study quantified the association of mpox infection in the first 100 days of the outbreak with a recent history of a nationally reportable sexually transmitted infection (STI), current use of pre-exposure prophylaxis for HIV (PrEP), and HIV diagnosis among gay, bisexual, and other men who have sex with men (GBMSM).

**Methods:** A case-control study was conducted among 229 GBMSM aged 18 years or older tested for mpox in a large, diverse safety-net health system in Denver, Colorado between May 25, 2022 and September 1, 2022. Dates of syphilis treatment and laboratory confirmed gonorrhea and chlamydia were used to determine whether an STI occurred in the preceding year, 6 months, or concurrently with ±30 days the mpox test. Patients with current PrEP use were identified through electronic health records, medication dispensation dates, and negative HIV serostatus.

**Results:** A larger proportion of cases than controls were over the age of 35, non-White, used PrEP, and were not living with HIV. Mpox infection was not associated with HIV diagnosis (odds ratio 0.62, 95% CI 0.35-1.11). After adjusting for age group and race/ethnicity, an STI in the preceding year increased the odds of mpox infection by 82% (adjusted odds ratio [aOR] 1.82, 95% CI 1.02-3.24) and an STI in the preceding 6 months increased the odds by 100% (aOR 2.00, 95% CI 1.10-3.48). Patients with mpox infection were 2.4 times more likely to have a concurrent STI compared to those without mpox (aOR 2.40, 95% CI 1.34-4.30). Among those without a HIV diagnosis, current PrEP use increased the odds of mpox infection by 100% (aOR 2.30, 95% CI 1.13-4.70).

**Conclusion:** This analysis demonstrated that there is a temporal relationship between mpox positivity among GBMSM and a recent history of STI as well as current PrEP use. These results offer several actionable implications. For healthcare providers, it is crucial that routine STI testing be offered when testing for mpox given the association between mpox infection and a concurrent STI. For public health agencies, this analysis suggests that mpox vaccine eligibility be universally expanded to include GBMSM with current PrEP for HIV use. Finally, health systems positioned to do so should consider proactively identifying GBMSM with a recent history of STI and current PrEP use for vaccine outreach.

**CRUDE ASSOCIATIONS BETWEEN MPXV INFECTION AND SEXUALLY TRANSMITTED INFECTION, PRE-EXPOSURE PROPHYLAXIS USE, AND HIV DIAGNOSIS**

<table>
<thead>
<tr>
<th>STI &amp; PrEP</th>
<th>Cases (n=229)</th>
<th>Controls (n=1,012)</th>
<th>OR (95% CI)</th>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2.02 (1.35-3.01)</td>
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<tr>
<td>No</td>
<td>204 (90.0)</td>
<td>967 (95.5)</td>
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<tr>
<td>Current PrEP</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47 (20.6)</td>
<td>36 (3.6)</td>
<td>2.08 (1.31-3.30)</td>
</tr>
<tr>
<td>No</td>
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<td>976 (96.4)</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>34 (3.4)</td>
<td>1.79 (1.33-3.32)</td>
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<td>978 (96.6)</td>
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</tr>
<tr>
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<td></td>
<td></td>
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<tr>
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<td>21 (2.1)</td>
<td>2.08 (1.34-3.47)</td>
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<td>991 (97.9)</td>
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<tr>
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</tbody>
</table>

**1034 HIV AND SEXUALLY TRANSMITTED INFECTIONS AMONG MPXV CASES, TEXAS 2022**

Analise Monterosso, Kacey Russell, Enyinnaya Merengwa, Kristyn Krolkowski, Abigail Jordan, Kenneth Davis, Rania Milleron, Stephen White

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**Background:** Syphilis, Chlamydia, Gonorrhea (STD) and HIV are reportable conditions in Texas. Men who have sex with men (MSM) and racial minority populations are disproportionately impacted by these conditions. The first mpox case in Texas was identified on June 7, 2022, and the U.S Department of Health and Human Services declared mpox a public health emergency on August 4, 2022. Due to the disproportionate impact of mpox on the same populations, Texas Department of State Health Services (TDSHS) began conducting routine HIV/STD/mpox matches in September 2022.

**Methods:** The data match was conducted with all persons reported to the TDSHS Emerging and Acute Infectious Disease Unit with confirmed and probable mpox. Data elements and person level identifiers reported through routine electronic reporting were used for the match, including first and last name, date of birth, sex at birth, and patient address. Mpox cases reported to TDSHS thru November 28, 2022, were matched to the HIV registry and the STD registry. All persons living with HIV/AIDS (PLWHA) were included in the HIV dataset, and all persons diagnosed with an STI within the previous 12 months were included in the STD dataset. Match processes were completed using SAS 9.4 and Link Plus.

**Results:** Of the 2,826 mpox cases reported to TDSHS thru November 28, 2022, 1,413 (50%) matched to the HIV case registry. Among the 2,826 mpox cases, 1,945 (69%) were either PLWHA or diagnosed with an STI. Men were disproportionately impacted by mpox, 2,733 cases (98%), compared to women, 93 cases (3%). There are large differences in the rate of coinfection with HIV/STDs, 70% of men (n=1,920) diagnosed with mpox had an HIV/STD coinfection, while only 27% of women (n=25) had a coinfection. Among people in the 50-59 age range diagnosed with mpox, 73% had a coinfection (n=163), followed by the 30-39 age range at 72% (n=845). Among PLWHA and mpox cases, 1,323 (94%) had a documented risk history of sex with gay or bisexual men. Black and African American people living with HIV were more likely to be diagnosed with mpox 594 (61%) compared to White (42%) or Hispanic (47%) PLWHA.

**Conclusion:** Even as mpox, HIV and STDs disproportionately impact MSM and racial minority populations, the distribution of that impact is not uniform across age, sex, race or geographic regions of the state. Prevention for all diseases should be focused on populations experiencing higher burden of disease.
Conclusion: Although concurrent STIs among patients with mpox were common when testing was performed, most patients tested for mpox in our health system were not comprehensively tested for STIs. Electronic health record-based strategies to promote concurrent mpox/STI testing are needed.

1036 WITHDRAWN

1037 INEQUITIES IN TRAVEL-TIME TO HIV TREATMENT IN AFRICA: A 3 COUNTRY COMPARISON
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Background: UNAIDS has recently announced a new strategy “End Inequalities. End AIDS. Global AIDS Strategy 2021-2026”. Increased travel-time to health facilities has been associated with decreased treatment initiation and retention. We analyze data from the nationally representative Population-Based HIV Impact Assessment (PHIA) surveys from Eswatini, Malawi, and Zambia to identify inequities in travel-time to treatment.

Methods: Data were collected in 2015/16 in Malawi, and 2016 in Eswatini and Zambia. The PHIAs collect questionnaire data and blood samples (used to identify the HIV status of each individual and detect the presence of antiretrovirals). People living with HIV (PLHIV) who reported being on antiretrovirals were asked to specify travel-time to receive treatment: < 1 hour, 1 to 2 hours, or > 2 hours. We first determined epidemic severity, and treatment coverage among adults aged 15-59. We then fit the observed travel-time data using Logistic cumulative distribution functions.

Results: Eswatini has the most severe epidemic: 28% prevalence, similar in urban (30%) and rural (27%) areas. Prevalence was 11% in Malawi, 12% in Zambia, and higher in urban than rural areas (14% to 10% [Malawi], 15% to 9% [Zambia]). Zambia has a predominantly urban epidemic: 58% of PLHIV in urban areas. Malawi and Eswatini have predominantly rural epidemics: 63% and 69% of PLHIV in rural areas. Eswatini had 77% treatment coverage, Malawi 70%, and Zambia 62%. Both Eswatini and Malawi had higher coverage in rural than urban areas: 78% vs. 73%, 71% vs. 66%. In Zambia, coverage was higher in urban (66%) than rural (57%) areas. The cumulative proportion of PLHIV on treatment is an increasing function of travel time (Figure); on average, PLHIV in Eswatini had the shortest travel time, PLHIV in Malawi had the longest. A fairly high percentage of patients traveled over 2 hours for treatment: 21% in Zambia, 19% in Malawi, and 11% in Eswatini. In all three countries, we found substantial urban-rural differences: a greater proportion of patients in rural areas, in comparison with urban areas, travelled for over 2 hours. Even in urban areas, quite a few traveled for over 2 hours to reach treatment: 11% (Zambia), 10% (Malawi), and 6% (Eswatini).

Conclusion: We have identified substantial inequities in access to treatment in Malawi, Eswatini, and Zambia when coverage levels were fairly high. As coverage has increased, it is important to determine whether inequities still exist and, if so, identify strategies to eliminate them.

Differences in travel time: country-specific and urban-rural

1038 EVALUATION OF MEDICAL DRONES FOR ANTI-RETROVIRAL DELIVERY IN AN ISLAND POPULATION
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1Makerere University College of Health Sciences, Kampala, Uganda, 2Infectious Diseases Institute, Kampala, Uganda, 3Janssen Research and Development, Beerse, Belgium, 4Johnson and Johnson, Narobi, Kenya, 5Infectious Disease Institute, Kampala, Uganda

Background: The Kalangala district is comprised of 64 islands in Lake Victoria. These island-dwelling communities have the highest HIV prevalence (27%) and lost to follow up from HIV care (50%) in Uganda. Delivery of anti-retro viral therapy (ART) is a challenge due to the geography and the nomadic nature of the community. In September 2021 we commenced delivery of ART to people living with HIV (PLHIV) using unmanned aerial vehicles (medical drones).

Methods: This study evaluated the feasibility of medical drone ART delivery to peer support groups as part of differentiated service delivery (DSD) implementation. Two DJI Matrice 300 drones flew from the island location of Bufumira health centre (BHC) to 5 remote landing sites previously receiving ART through outreachs by boat. The impact on PLHIV was assessed using surveys to PLHIV at baseline and those who received ART by drone in the 12th month after ART delivery commenced. PLHIV at Mazinga Health Centre (MHC) on another
island were interviewed as a control group. Routinely collected data from BHC and MHC was analysed.

Results: 150 PHLV at BHC and 100 at MHC were interviewed at baseline. In September 2021, medical drone flights delivery commenced to 43 PHLV on three landing sites served by BHC. Each delivery carried 7 bottles of drug tablets at 9.3 min, with an average speed of 6.6 km/h. At 12 months there was

1039. DOES TYPE OF PICK-UP POINT INFLUENCE 12-MONTH VIROLOGIC SUPPRESSION IN SOUTH AFRICA?

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1Massachusetts General Hospital, Boston, MA, USA, 2AIDS Healthcare Foundation, Durban, South Africa, 3RAND Corporation, Los Angeles, CA, USA

Background: South Africa’s Central Chronic Medicine Dispensing and Distribution (CCMDD) Program allows people living with HIV who are clinically stable the choice of pick-up point to increase convenience. Our objective was to assess the impact of community pick-up (vs. clinic-based ‘fast-track’ pick-up) on rates of virologic suppression 12 months after CCMDD enrolment.

Methods: We enrolled an observational cohort of adults (≥18 years) who met CCMDD clinical eligibility criteria (not pregnant, on ART for ≥1y, and virologically suppressed) in 7 public sector clinics in Umlazi, KwaZulu-Natal. We assessed CCMDD effectiveness using virologic suppression at 12 months (range 6-18 months) following CCMDD enrolment using data from the National Health Laboratory Service database. In addition to age and gender, we identified other potential predictors of virologic suppression in univariate models with p < 0.2 and fit a multivariable logistic regression model.

Results: Among 1642 participants, 67% were female, with median age 36 years (IQR 44-76), and median duration on ART prior to CCMDD enrolment of 2 years (IQR 1-5). 919 (56%) opted for community ART pick-up at enrollment. Among 1289 with viral load data available, 1112 (86%) were virologically suppressed at one year. In addition to type of pick-up point, variables included in the multivariable model were year of HIV diagnosis, distance to clinic, baseline self-efficacy, and HIV treatment beliefs. No predictors of virologic suppression were statistically significant. In particular, opting for clinic-based pick-up point (vs. community-based) was not associated with achieving 12-month virologic suppression (aOR 1.12, 95 CI 0.79-1.58).

Conclusion: In this multi-site study of the CCMDD program in South Africa, choice of pick-up point (community- vs clinic-based) was not associated with virologic suppression 12 months after enrollment in the CCMDD program. These results suggest that provision of community-based ART pick-up points has not reduced continued viral suppression in a population enrolled in the CCMDD program.

1040. AN ADAPTIVE INTERVENTION TRIAL TO SUPPORT VIRAL SUPPRESSION AMONG FEMALE SEX WORKERS

Shereen Schwartz1, Carly Comins1, Mfezi McInlgana,2 Siyaphambili trial tested two adaptive interventions to identify efficient pathways to viral suppression, including a nurse-led decentralized treatment program (DTP) using a mobile van vs. FSW-peer led individualized case management (ICM) consisting of face-to-face visits and calls.

Methods: Non-pregnant, adult disengager FSW living with HIV were recruited by TB HIV Care at its drop-in center and FSW venues in Durban, South Africa for a sequential multiple assignment randomized trial (SMART). Non-virally suppressed (≥50 copies/mL) FSW were randomized to DTP vs. ICM interventions, then re-randomized at 6-months per viral load responsiveness to continue vs. receive both interventions if non-virally suppressed, or continue vs. return to standard care (SOC) if virally suppressed. The re-randomized strategy was continued until 18-months; the primary endpoint was an intention-to-treat 18-month combined retention and viral suppression outcome in DTP vs. ICM.

Results: Overall, 1391 FSW were enrolled from June 2018-March 2020 (median age 31 [IQR 27-37]); 519/1391 (38%) were virally suppressed at enrollment. 777 non-virally suppressed FSW were randomized across the adaptive interventions. At 18-months, 117/777 (15.1%) of FSW were retained and virally suppressed.
There was no difference across arms, 16.0% DTP vs. 14.1% ICM (p=0.455); nor in those stepping up or stepping down interventions (Figure). Among FSW with sustained viremia at 6- and 12-months who returned for further bloodwork, 74/80 (92.5%) were resistant to first-line ART. In a non-randomized TB HIV Care program comparator arm of non-virally suppressed FSW engaged in HIV care during the enrollment period, 4/86 (5%) were retained and virally suppressed at 18-months.

Conclusion: No differences in treatment outcomes were observed between study arms. However, FSW who responded early were the most likely to be suppressed, even if stepped down after six months to the standard of care. While outcomes appear modestly improved over SOC, they are extremely suboptimal and high rates of drug resistance were observed. These results reinforce the urgent need to improve treatment outcomes among FSW in South Africa to end new HIV infections by 2030.

Siyaphambili Study Design and Outcomes

Siyaphambili SMART Trial
Study design

1041 TRENDS IN PERSISTENT HIV VIREMIA DURING UNIVERSAL TEST-AND-TREAT SCALE-UP IN UGANDA
Joseph G. Rosen1, Larry William W. Chang2, Ronald M. Galwango2, Robert Ssekubugye2, Anthony Ndyなもの1, Gertrude Nkwigwa2, Katherine B. Rucinski3, Caitlin E. Kennedy1, Fred Nalugoda3, Godfrey Kigozi3, Joseph Kagaayi3, Thomas C. Quinn4, Lisa A. Mills5, Steven J. Reynolds2, Mary K. Grabowski2, Caitlin E. Kennedy1, Fred Nalugoda3, Godfrey Kigozi3, Joseph Kagaayi3, Thomas C. Quinn4, Lisa A. Mills5, Steven J. Reynolds2, Mary K. Grabowski2

1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3Rakai Health Sciences Program, Entebbe, Uganda, 4National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, 5Centers for Disease Control and Prevention, Kampala, Uganda

Background: Universal Test and Treat (UTT) has expanded antiretroviral therapy (ART) access globally, but population-based studies of longitudinal HIV treatment outcomes following UTT are rare. We characterized population-level trends in durable HIV viral load suppression (VLS) and persistent and intermittent viremia after UTT scale-up in Rakai, Uganda.

Methods: We ascertained viral load status among Rakai Community Cohort Study participants in 4 hyperendemic fishing villages and 36 lower-prevalence inland communities across 3 survey rounds (2015 to 2020), coinciding with UTT rollout. For each participant, viral load was assessed over 2 study visits (i.e., visit-pair, ~1.8-year interval between visits), and classified as follows: persistent viremia (~1,000 copies/ml across visits), durable VLS (< 1,000 c/ml across visits), new/renewed VLS (~1,000 c/ml at index visit only), or viral rebound (~1,000 c/ml at follow-up only). Prevalence estimates for viral load outcomes in each visit-pair were assessed over calendar time and by age and gender. Individual- and community-level predictors of persistent HIV viremia were also identified using multivariable robust Poisson regression with generalized estimating equations, reported as adjusted risk ratios (aRR) with 95% confidence intervals (95%CI).

Results: Overall, 3,080 persons (mean age: 34 years, 62% women) contributed 4,604 visit-pairs to the analysis. One-fourth of visit-pairs exhibited any viremia, of which 42% were persistently viremic, and 10% had viral rebound. Among visit-pairs with persistent viremia, 21% were in persons reporting ART use for >1 year. Significant increases in durable VLS prevalence over time (71% to 90% across communities) were observed. Among persons with non-virally suppressed viremia (12% to 8%), however, the magnitude of these declines varied substantially across communities (Figure 1). Persistent viremia was significantly higher among youth (~29 vs. 40–49 years: aRR 2.96, 95%CI 2.21–3.96), men (aRR 2.40, 95%CI 1.87–3.07), and individuals reporting inconsistent condom use with non-stable partners (aRR 1.38, 95%CI 1.10–1.74) and hazardous alcohol use (aRR 1.09, 95%CI 1.03–1.16).

Conclusion: Durable VLS increased with UTT, but 42% of viremic visit-pairs, nearly one-fourth of which were in persons on ART, remained unsuppressed for >1 year. Given the concentration of viremia in groups with known barriers to HIV care and elevated risks of onward HIV transmission, UTT alone may be insufficient to optimize clinical outcomes and achieve HIV epidemic control in this setting.

Community-level prevalence of persistent HIV viremia, by calendar period

1042 USAGE PATTERNS AND OUTCOMES IN A LARGE COMMUNITY ART PROGRAMME IN SOUTH AFRICA
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1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2University of Oxford, United Kingdom

Background: Differentiated antiretroviral therapy (ART) delivery programmes are being rolled out globally but there is little data on long-term implementation and outcomes for people living with HIV (PLHIV). We describe patterns of exposure to community ART delivery and associated treatment outcomes in the South African Centralized Chronic Medicines Dispensing & Distribution programme over 3.5 years.

Methods: We performed a retrospective cohort study among PLHIV on first-line ART who were referred for community ART delivery between Oct 2016 – Mar 2019 from 56 clinics in KwaZulu-Natal, South Africa. Follow up ended in Mar 2020. We used group-based trajectory modelling to characterize patterns of exposure to community ART following referral, and survival analyses to measure the association between time-varying exposure to community ART and time to loss-to-care, defined as no clinic visit for >365 days. We used logistic regression with generalized estimating equations to quantify the association between proportional exposure to community ART 12 months prior to viral load measurement and viraemia (>50 cps/mL) among those in care. Models of loss-to-care and viraemia adjusted for baseline patient age, gender and time on ART.

Results: Among the 37,596 patients referred to community ART, 68.6% were female with a median (interquartile range (IQR)) age of 38 (32-45) years, and 99.3% were on tenofovir-based regimen. Median (IQR) follow-up time was 656 (430-841) days. Time spent in community ART varied during follow-up; ~40% remained consistently in community ART following referral, ~20% returned to clinic-based care after their first referral visit while the remaining 40% oscillated between community ART and clinic-based care. The incidence of loss-to-care was 4.46 per 100 person-years and community ART exposure was associated with a 41.1% (95% confidence interval (CI): 34.1%–47.3%) reduction in the hazard of loss-to-care after adjusting for other covariates. Among those with at least one viral load measured after referral (N=33,378), 5.4% became viraemic. A 10% increase in the proportion of time spent in community ART in the 12-month period before viral load measurement was associated with a 4% (95% CI: 2%-5%) reduction in the adjusted odds of viraemia.

Conclusion: Community ART exposure patterns vary considerably after referral into the programme. Promoting consistent programme adherence will reduce clinic burden and the likelihood of patients being lost to care, whilst sustaining viral suppression.
HIGH COVERAGE OF HIV TREATMENT, LOW COVERAGE OF PREVENTION SERVICES AMONG KP IN INDIA
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Background: Over the past decade, India has greatly expanded access to HIV services such as testing and treatment, resulting in declines in general population prevalence. However, key populations continue to experience a high burden of HIV and may have unique challenges with accessing critical HIV services.

Methods: We used respondent-driven sampling (RDS) to separately recruit members of key populations (KP) – men who have sex with men (MSM), transgender persons (TG), and female sex workers (FSW) – in Pune, India. People were eligible to enroll if they were a KP member, ≥18 years, and had a valid RDS referral coupon. Study participants completed rapid onsite HIV testing and completed an interviewer-administered survey covering demographics, behaviors, and health service utilization. By KP, we describe the uptake of HIV prevention, care, and treatment services. We assessed correlates of HIV testing in the prior 12 months (excluding known positives) and diagnosis of HIV infection (among all those living with HIV (PLHIV)) using logistic regression.

Results: As of September 13, 2022, we recruited 387 MSM, 432 TG, and 225 FSW. Median age was 28 (MSM), 26 (TG), and 40 years (FSW). HIV prevalence was 13% (MSM), 5% (TG), and 22% (FSW). Among negative KPs, FSW had high utilization of free condoms (94%) and HIV testing (85%) compared to MSM and TG (Figure). TG were most likely to be aware of PrEP (21%) but use was negligible across all KP. For HIV outcomes among PLHIV, 83% MSM, 78% TG, and 96% FSW were previously diagnosed. Current ART use was relatively high – 80% (MSM), 64% (TG), and 93% (FSW) but a history of viral load testing was low (44% MSM, 29% TG, 32% FSW) and many did not know the result. Among PLHIV, 33% (MSM), 56% (TG), and 24% (FSW) had been offered partner testing. Correlates of recent HIV testing included older age, higher education, and being FSW or TG. Correlates of diagnosis were older age and residing in the district for more than 5 years.

Conclusion: Treatment coverage among PLHIV is approaching UNAIDS targets, however utilization of prevention services is low, alongside high HIV prevalence among most KP. This leads to missed opportunities – particularly in HIV testing – to identify untreated PLHIV and reduce ongoing transmission. HIV prevalence, care continuum, and prevention service utilization among key populations in Pune, India

ACHIEVING THE EHE INCIDENCE REDUCTION GOALS AMONG AT-RISK POPULATIONS IN THE SOUTH
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1University of Washington, Seattle, WA, USA, 2Centers for Disease Control and Prevention, Atlanta, GA, USA, 3Emory University, Atlanta, GA, USA

Background: Antiretroviral therapy (ART) coverage remains sub-optimal in much of the United States, particularly the Southern region, and Non-Hispanic Black or African American persons (NHB) continue to be disproportionately impacted by the HIV epidemic. The “Ending the Epidemic in the U.S.” (EHE) initiative seeks to reduce HIV incidence nationally by focusing resources towards the most highly impacted localities and populations. This study evaluates the impact of hypothetical improvements in ART and preexposure prophylaxis (PrEP) coverage to estimate the levels of coverage needed to achieve EHE goals in the South.

Methods: We developed a stochastic, agent-based network model of 500,000 individuals to simulate the HIV epidemic and hypothetical improvements in ART and PrEP coverage.

Results: New infections declined by 78.6% at 90%/40% ART/PrEP and 94.3% at 100%/50% ART/PrEP from 2022-2030. Declines in annual incidence rates surpassed 75% by 2025 with 90%/40% ART/PrEP and 90% by 2030 with 100%/50% ART/PrEP coverage. Increased ART coverage among NHB gay, bisexual, and other men who have sex with men (MSM) was associated with a linear decline in incidence among all MSM. Declines in incidence among Hispanic/Latino and White/Other MSM were similar regardless of which MSM race group increased their ART coverage, while the benefit to NHB MSM was greatest when their own ART coverage increased. The incidence rate among NHB women declined by over a third when either NHB heterosexual men or NHB MSM increased their ART use respectively. Increased use of PrEP was associated with a decline in incidence for the groups using PrEP. MSM experienced the largest absolute declines in incidence with increasing PrEP coverage, followed by NHB women.

Conclusion: Our analysis indicates that it is possible to reach EHE goals. The largest reductions in HIV incidence can be achieved by increasing ART coverage among MSM and all race groups benefit regardless of differences in ART initiation by race. Improving ART coverage to > 90% should be prioritized with a particular emphasis on reaching NHB MSM. Such a focus will reduce the largest number of incident cases, reduce racial HIV incidence disparities among both MSM and women, and reduce racial health disparities among persons with HIV. NHB women should also be prioritized for PrEP outreach.

PROGRESS ON UNAIDS 95-95-95 TARGETS AMONG KEY POPULATIONS IN 3 TANZANIA REGIONS
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Background: Key populations (KP) such as female sex workers (FSW) and people who inject drugs (PWID) have high burden of HIV. The Tanzania HIV Impact Survey 2016-2017 estimated that 61% of people living with HIV (PLHIV) in the general population were aware of their status, 94% of diagnosed PLHIV were on antiretroviral therapy (ART), and 87% of ART clients achieved viral load suppression (VLS). UNAIDS estimates that 88% of PLHIV are now aware of their status in Tanzania in 2021; however, estimates of these targets among KP are unavailable. We measured progress towards the UNAIDS 95-95-95 targets among FSW and PWID in Mwanza, Mbeya, and Dodoma regions of Tanzania.

Methods: We conducted a bio-behavioral survey (RBS) using respondent-driven sampling (RDS) between March and May 2022. HIV serostatus among participants was determined based on the national HIV testing algorithm. HIV viral load was measured for HIV-positive participants using plasma samples. In RDS-adjusted analysis, we calculated the proportion of HIV-positive FSW and PWID who were aware of their status, diagnosed FSW and PWID who received ART, and ART clients with viral load suppression (VLS). UNAIDS estimates that 88% of PLHIV are now aware of their status in Tanzania in 2021; however, estimates of these targets among KP are unavailable. We measured progress towards the UNAIDS 95-95-95 targets among FSW and PWID in Mwanza, Mbeya, and Dodoma regions of Tanzania.

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Conclusion: Our findings suggest that HIV burden continues to be disproportionate among FSW and PWID compared with the general population in the study regions. Substantial progress has been made in diagnosing HIV among FSW and PWID. Targeted and enhanced outreach is needed to reach the first 95 target. The second and third 95 targets have been met or exceeded suggesting that once KP know their status, with appropriate services, they can and do access ART and achieve VLS.

1047 REACHING THE THIRD 95 IN UGANDA AFTER LOWERING HIV VIRAL LOAD SUPPRESSION CUTOFFS
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Background: Per UNAIDS 95–95–95 Fast-Track targets, reaching the “third 95” requires 86% of people living with HIV to achieve viral load suppression (VLS), defined by UNAIDS and WHO as < 1,000 HIV RNA copies/ml. Emerging evidence linking persistent lower-level viremia to HIV drug resistance has prompted calls to redefine VLS using lower cutoffs. However, it is unclear how lowering VLS cutoffs might impact achievement of the Fast-Track targets or resources required for delivering HIV treatment.

Methods: We used data from a prospective population-based survey in rural south-central Uganda, the Rakai Community Cohort Study, to assess the impact of lowering VLS cutoffs on estimated progress towards the 95–95–95 VLS target. We estimated VLS by calculating the proportion of serologically confirmed HIV cases with undetectable plasma viral loads or exhibiting viremia below specific cutoffs measured over 3 survey rounds in 40 communities (2015 to 2020). We ascertained differences in population VLS estimates across 4 routinely used VLS cutoffs: < 1,000, < 400, < 200, and < 50 copies/ml. We also assessed the relative increase in the fraction of viremic persons living with HIV if VLS cutoffs were lowered.

Results: Overall, 5,814 people living with HIV (mean age: 33 years, 63% women) contributed 10,418 observations to the analysis. In the final survey (2018–2020), estimated population VLS (86%, 95% confidence interval [95%CI] 85–87%) met the 95–95–95 Fast-Track target using a cutoff of < 1,000 copies/ml (Figure 1A). However, when using the more conservative cutoffs of < 200 and < 50 copies/ml, population VLS fell to 84% (95%CI 83–85%) and 76% (95%CI 74–77%), respectively. Among persons with detectable viral load, lowering the VLS cutoff from < 1,000 to < 200 copies/ml increased the proportion of people living with HIV who were viremic by 17% (Figure 1B).

Conclusion: Lowering VLS cutoffs may substantially impact Fast-Track target achievement, requiring countries and subnational units to reframe progress towards HIV epidemic control goals. It may also markedly increase programmatic resources needed for the clinical management of persons living with HIV who exhibit lower-level viremia.

Viral load suppression prevalence (Panel A) and the distribution of viral copy counts among viremic (>50 copies/ml) persons living with HIV, by calendar period.
1049 EARLY CASE-FINDING IS STILL NEEDED IN THE HIV TEST-AND-TREAT ERA TO PREVENT MORTALITY

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Background: Universal test and treat (UTT) for all people living with HIV (PLHIV) is key to HIV epidemic control. Early case-finding is still needed in the HIV test-and-treat era to prevent mortality.

Methods: We carried out a household-randomized, controlled trial to evaluate the effect of the norming strategy among household contacts of pulmonary TB patients in Kampala, Uganda (ClinicalTrials.gov #NCT05124665). Community health workers (CHW) visited homes of TB index patients to screen contacts of patients with TB, for whom HIV greatly increases the risk of incident TB and death.

Results: Monthly death, interruptions in treatment and transfer-out among patients with TB, for whom HIV greatly increases the risk of incident TB and death. However, up to half of household contacts decline HIV test offers during contact investigation. We evaluated a brief social-behavioral norming strategy to increase acceptance of HIV testing during TB household contact investigation compared to standard strategies.

Methods: CS system is a repository of sentinel events among PLHIV (HIV diagnosis, linkage and retention on ART) derived from the Kenya National Data Warehouse (NDW) of electronic medical record containing data from over 80% of PLHIV on ART. We extracted data on outcomes in the first 12 months after enrollment among PLHIV enrolled in care between January to December in 2020 (97,176) and 2021 (84,103). Outcomes were: death, IIT, transfer-out (TO), and active on care. Patients who had IIT but not return for a clinic visit or ART refill for more than 90 days from the last scheduled visit, TO was a client who transferred out of a facility without a record of transfer-in (TI) to another facility. Patients with either a recorded death, IIT or who had a recorded TI after TO during the follow-up period were active on care. We used descriptive statistics to calculate monthly average and cumulative death, IIT and TO, accounting for censoring at the end of each month.

Results: Monthly average rate of death, IIT and TO in the first 12 months from enrollment for each cohort are shown in figure 1. Monthly attrition was similar in the two cohorts. While death and TO rates were highest in the first month from enrollment, IIT rates peaked between months three and five. Cumulatively, 3,044 (3.3%) and 2,859 (3.7%) of clients died in the first year of enrollment in 2020 and 2021, respectively. IIT accounted for the greatest cumulative rate of attrition in both cohorts. In the 2020 and 2021 cohorts, 19.1% (15,046) and 27.6% (14,021) of clients experienced treatment interruption, respectively. Cumulative rates of TO were 7.8% (6273) in 2020 and 5.0% (3.684) in 2021.

Conclusion: HIV CS data in Kenya shows high levels of attrition in the first 12 months of enrollment, with concerning trends in mortality and IIT. These findings suggest early diagnosis of HIV, before the onset of advanced disease, with intensive clinical management and adherence support to PLHIV newly initiating ART continue to be key areas for program strengthening. Examination of causes of death and IIT could identify additional areas for program improvement.

Figure 1: Monthly death, interruptions in treatment and transfer-out among PLHIV who initiated ART in 2020 and 2021, Kenya National HIV Case Surveillance System.
Results: 328 contacts in 99 index patient households were randomized to the intervention and 224 contacts in 66 index patient households were randomized to the standard-of-care arm. In the intervention arm, 285 (87%) contacts met eligibility criteria. In the control arm, 187 (92%) contacts met eligibility criteria. Completion of HIV testing was higher in the intervention arm (98% versus 92%, difference +6%, 95%CIs +2% to +10%, p=0.004). Yield of HIV testing was 2.1% in the intervention arm and 0.6% in the control arm (p=0.22).

Conclusion: A norming intervention significantly improved uptake of HIV testing among household contacts of patients with TB.

1051 MODELED IMPACT OF HIV SELF-TESTING FOR PrEP SCALE-UP ON DRUG RESISTANCE IN KENYA
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Background: Community-based oral pre-exposure prophylaxis (PrEP) provision has the potential to expand PrEP delivery; however, HIVST can have lower field performance than standard provider-administered testing, potentially leading to inappropriate PrEP provision to persons with HIV and contributing to the development of drug resistance. The impact of using HIVST in PrEP scale-up is not well understood.

Methods: We parameterized an agent-based network model, EMOD-HIV, to project the impact of PrEP scale-up in western Kenya using either 1) provider-administered nucleic acid technique (NAT), 2) provider-administered rapid diagnostic tests detecting antibodies (Ab RDT), 3) capillary whole blood-based HIVST, or 4) oral-fluid HIVST, compared with a counterfactual of no PrEP. We assumed individuals 18-49 years entering a heterosexual partnership had a 75% probability of initiating PrEP (and continuing quarterly thereafter).

Results: In all HIV testing scenarios, the average estimated PrEP coverage was 29%, which was projected to avert 50% of HIV infections and 14% of HIV-related deaths over 20 years. Of an estimated 45 million PrEP initiations, the number of individuals with acute HIV infection who were inappropriately initiated on PrEP were 4,028 and 4,488 in the blood and oral HIVST scenarios respectively, compared to 988 in the Ab RDT and 92 in the NAT scenarios. The number of individuals with chronic HIV inappropriately initiated on PrEP were 7,645 and 13,653 in the blood and oral HIVST scenarios respectively, compared to 1906 in the Ab RDT and 141 in the NAT scenario. HIV infections with PrEP-associated nucleoside reverse transcriptase inhibitor (NRTI) resistance were 0.5% and 0.7% in the blood and oral HIVST scenarios respectively, compared to 0.2% and 0.1% in the Ab RDT and NAT scenario, respectively. Accounting for background NTRI resistance, we found similar proportions of resistance across all scenarios (1.3% compared to 1.4% in the no PrEP scenario).

Conclusion: Increasing PrEP coverage has the potential to avert half of new HIV infections over 20 years. We project a low number of persons with HIV inappropriately initiated on PrEP due to the small number of acutely infected individuals. The population prevalence of NRTI resistance was similar across scenarios, largely due to the reduction in HIV (and thus HIV-related drug resistance) in the PrEP scenarios compared to the counterfactual of no PrEP.

1052 PREDICTORS OF PLWH NEWLY PRESENTING TO CARE WITH VIRAL SUPPRESSION IN LUSAKA, ZAMBIA
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Background: As HIV treatment access in sub-Saharan Africa has expanded, it has become increasingly important to follow people living with HIV (PLWH) across multiple healthcare facilities to fully describe their HIV care journey. In Zambia, following PLWH across HIV care sites may be complicated by silent transfer. As part of a parent study on recent HIV infection, we measured viral load at baseline among PLWH newly presenting to care at two high-volume clinics in Lusaka, Zambia, and assessed predictors of potential silent transfer.

Methods: We conducted this sub-study using socio-demographic, clinical, and behavioral data from a cohort enrolled in the parent study from 2 June 2021–24 February 2022. We measured baseline HIV viral load as part of the national recent infection testing algorithm, and, for this analysis, used results to identify and describe PLWH newly presenting to care with viral suppression (i.e., potential silent transfers). We used mixed effects Poisson regression models to estimate prevalence ratios for viral suppression (at ≤1,000 copies/ml and ≤50 copies/ml) at presentation to care, and to construct marginal probability estimates by age category and sex.

Results: We included 248 individuals among whom 66 (27% [66/248]) had viral suppression defined at ≤1,000 copies/ml and 53 (21% [53/248]) at ≤50 c/ml thresholds, respectively. Participants reporting widowed marital status had a significantly lower adjusted prevalence of baseline viral suppression (adjusted prevalence ratio [aPR]=0.28; 95% confidence interval [CI]:0.10, 0.83) compared to married participants, while participants age 40+ years had a significantly higher adjusted prevalence of suppression (aPR:2.10; 95% CI:2.08, 2.13) compared to participants aged 18-24 years. Participants reporting no formal education had a significantly higher adjusted prevalence of baseline viral load ≤1000 c/ml (aPR:1.63; 95% CI:1.52, 1.75). Finally, adjusted marginal probability for potential silent transfer was highest among older (40+ years) females (41.8%, 95% CI:39.3, 43.3%).

Conclusion: With a high proportion of PLWH newly presenting to care with baseline viral suppression, silent transfer is potentially a major issue in routine HIV programs. Our observations suggest characteristics of patients who may engage in silent transfer, allowing for program improvement to better identify and support these PLWH.

Figure 1: Marginal probability of new client being suppressed at presentation to HIV care by sex. Note: estimates based on model adjusted for marital status, educational attainment, month of testing, and facility; CI – confidence interval

1053 LATE PRESENTATION IN OLDER PWH IS ASSOCIATED WITH MORTALITY AND POOR IMMUNE RESPONSE
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Background: HIV late presentation is increasingly observed among older people, although their healthcare access may be easier versus younger people. Data are limited regarding the burden of late presentation in older people in resource-limited settings, including whether they are at risk of poor HIV treatment outcomes.

Methods: We included people living with HIV (PWH) ≥15 years of age at ART initiation in the Thai National AIDS Program (NAP) from 2008 - 2019. Age and CD4 distributions in a 6-month window before ART initiation were assessed. Late presentation to care was defined as CD4 < 200 cells/mm3 at ART start. Immunological response (IR) was defined as achieving CD4 > 350 cells/mm3 after ART initiation. Death was confirmed by National death registry linkage. We used Cox regression to investigate the association of age at ART initiation, late presentation to care and other demographic characteristics with IR and all-cause mortality.

Results: A total of 286,933 PWH (39% female) were included. Median (IQR) age at ART initiation was 36 (28–43) years; 18%, 26%, 29% and 27% started ART during years 2008–2010, 2011–2013, 2014–2016 and 2017–2019, respectively. Median CD4 at ART initiation was 191 (IQR 67–363) cells/mm3. In 2019, the percent of late presentation was 26%, 42%; 49% and 52% in PWH aged 15-24, 25-34, 35-49 and ≥50 years, respectively. Median CD4 was lowest among PWH aged 25-34 years (203 (IQR 77-427) cells/mm3).

According to the analysis, older age was significantly associated with increased all-cause mortality (HR 1.13, 95% CI 1.09, 1.15) and decreased IR (HR 0.92, 95% CI 0.90, 0.94), after adjusting for sex, educational attainment, month of testing, and facility. In older PWH, CD4 ≥350 cells/mm3 was less frequently observed than younger age groups (55.6% compared to 62.3% in 18-24 years). In older PWH, median CD4 was lower than younger age groups (199 (IQR 67-418) cells/mm3 in ≥50 years, compared to 223 (IQR 79-457) cells/mm3 in 18-24 years).

Conclusion: Late presentation in older PWH is associated with increased mortality and poor IR. Older age was significantly associated with increased all-cause mortality and decreased IR, after adjusting for sex, educational attainment, month of testing, and facility. In older PWH, CD4 ≥350 cells/mm3 was less frequently observed than younger age groups (55.6% compared to 62.3% in 18-24 years). In older PWH, median CD4 was lower than younger age groups (199 (IQR 67-418) cells/mm3 in ≥50 years, compared to 223 (IQR 79-457) cells/mm3 in 18-24 years).

Acknowledgments: This study was funded by the World Health Organization's HIV/AIDS programme and the Thai National AIDS Program.
aged ≥50 years over the time (Figure 1B-F). Median time from ART start to IR was 12 (IQR 6-27) months. PWH aged ≥50 years at ART start had a lower risk of IR (adjusted hazard ratio [aHR]: 0.81, 95%CI 0.79-0.82) after adjusting for CD4 at ART start, sex, within country residential region, HIV stage, ART duration and type. Over 1,669,397 person-years of follow-up (PYS), 37496 (13%) died. The crude death rate was 2.25 (2.22-2.27)/100 PYS; the median of time from ART initiation to death was 1.9 (IQR, 0.5-4.5) years. Starting ART aged ≥50 years (aHR: 2.59, 95%CI 2.48-2.71 vs. 15-24 years) and late presentation (aHR: 4.02, 95%CI 3.81-4.24) were significantly associated with higher mortality risk.

Conclusion: Despite recent increases in HIV diagnoses among younger individuals, particularly young MSM in Thailand, the increasing proportion of older PWH who present late to care is concerning. The association of poor HIV outcomes in older PWH with late presentation signals an urgent need for implementation of targeted HIV testing to improve early diagnosis, and linkage to care for this vulnerable group.

Age and CD4 distribution at ART initiation

1054 POTENTIAL IMPACT OF LONG-ACTING ANTIRETROVIRAL THERAPY ON LOCAL HIV INCIDENCE

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Background: Long-Acting Antiretroviral Therapy (LAART) has demonstrated efficacy in virally suppressed people with HIV (PWH), and clinical trials are ongoing to evaluate its use in ART-naive and ART-experienced, unsuppressed PWH. LAART has the potential to reduce non-adherence and promote retention in HIV care, but its potential effects on local HIV epidemics remain unclear.

Methods: We used the Johns Hopkins HIV Epidemiologic and Economic Model (JHEEM), a model of local HIV epidemics, to simulate the potential impact of rolling out LAART in four US cities: Atlanta, Baltimore, Los Angeles, and Miami. We simulated LAART roll out at two levels for three separate indications, separately and in combination: (1) Transitioning either 25% or 50% of "durably suppressed" PWH (suppressed for ≥ 2 years on oral ART) onto LAART; (2) starting either 25% or 50% of ART-experienced PWH who were unsuppressed on LAART yearly; and (3) starting either 25% or 50% of ART-naive PWH on LAART (instead of oral ART).

All interventions ramped up from 2025 to 2028 and continued through 2035. We assumed LAART had equivalent efficacy to oral ART, but randomly allowed LAART to range from equally as likely to keep PWH engaged in care to twice as likely across simulations.

Our primary outcome was the reduction in the projected number of incident HIV infections from 2025 to 2035. Secondary outcomes included proportion of PWH in care at 12 months from ART start, sex, within country residential region, HIV stage, ART duration and type. Over 1,669,397 person-years of follow-up (PYS), 37496 (13%) died. The crude death rate was 2.25 (2.22-2.27)/100 PYS; the median of time from ART initiation to death was 1.9 (IQR, 0.5-4.5) years. Starting ART aged ≥50 years (aHR: 2.59, 95%CI 2.48-2.71 vs. 15-24 years) and late presentation (aHR: 4.02, 95%CI 3.81-4.24) were significantly associated with higher mortality risk.

Conclusion: Despite recent increases in HIV diagnoses among younger individuals, particularly young MSM in Thailand, the increasing proportion of older PWH who present late to care is concerning. The association of poor HIV outcomes in older PWH with late presentation signals an urgent need for implementation of targeted HIV testing to improve early diagnosis, and linkage to care for this vulnerable group.

1055 IDENTIFYING PREFERRED PROGRAM DELIVERY ATTRIBUTES FOR LONG-ACTING INJECTABLE ART

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Background: As long-acting injectable antiretroviral therapy (LAI-ART) enters clinical practice, it is crucial to incorporate patient preferences into the design of LAI-ART delivery programs, especially among underserved people living with HIV (PLWH).

Methods: Using formative qualitative research, we developed a tablet-based discrete choice experiment (DCE) for administration in three urban HIV clinics (UCSF’s Ward 86, Emory/Grady, University of Chicago), sampling for ≥100 patients per site and by care engagement status (less well engaged defined as detectable HIV RNA or no past-year HIV RNA with <2 primary care visits). The DCE presented two hypothetical LAI-ART programs over 10 choice sets, including an ‘opt-out’ option. Designs were characterized by six attributes: injection visit location, extra visit with your HIV doctor at the injection, extra support services, visit length, extended hours on nights/weekends, and cost. Analyses were performed using the Hierarchical Bayes model to estimate zero-centered part-worth utility scores across all 13 attribute levels and generate mean attribute relative importance scores (RIS). We also examined preferences by population segments: age, gender, race/ethnicity, sexual orientation, housing status, past-month substance use, and care engagement status.

Results: From December 2021-May 2022, 370 patients completed the DCE with median age 46, 34% cis-female/gender minority, 59% Black, 13% Latinx, 44% heterosexual, 34% homeless/unsuitable housed, 19% with substance use, and 27% less well engaged. Overall, cost (RIS 30.04%) and injection visit location (RIS 29.81%) were the most influential program design attributes, with preferred features being no cost and receiving injections at their HIV clinic. A cost of $40 and the mobile clinic option were the least preferred. Preferences for population segments were similar, except a slightly increased secondary preference for ‘place where you stay’ visit location was observed among those with substance use, ‘Other’ race/ethnicity, and the Emory site.

Conclusion: Assessing preferences of PLWH can inform a patient-centered approach to LAI-ART delivery. Among this predominantly racial/ethnic minority population, prioritizing that LAI-ART remains affordable and available at their HIV clinic could facilitate optimal uptake and delivery of this innovative treatment. However, sub-populations may further benefit from tailored approaches that provide injection visit location flexibility.
1056 UPTAKE AND OUTCOMES OF FIRST-LINE DOLUTEGRAVIR IN A LARGE SOUTH AFRICAN COHORT

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Background: South Africa has been introducing dolutegravir (DTG) into its large ART programme. We aimed to evaluate uptake and outcomes of first-line DTG, particularly in the context of initial safety concerns for women of child-bearing potential.

Methods: We analyzed de-identified, routine data from two separate cohorts at 59 primary care clinics in EThekwini Municipality, South Africa, from DTG introduction in Dec 2019 to Feb 2022. In the ‘initiator’ cohort, we used multivariable Poisson regression models with robust standard errors to evaluate the likelihood of being initiated on DTG vs non-DTG ART, and of attrition (loss to follow up/death) and viraemia >50 cps/mL, at 12 months post-initiation. For the ‘transition’ cohort we used multivariable Cox proportional hazards models to assess the transition from non-DTG to DTG-based first-line ART.

Results: Of 33,533 adults initiating ART, 21,362 (63.7%) were women, median age was 32 years (IQR 27-42). Overall, DTG initiation was lower in women vs men (adjusted risk ratio 0.77, 95% CI 0.75-0.79). This difference by gender was larger in younger people (e.g. 15-24 years: women vs men aRR 0.68, 95% CI 0.60-0.74), it was not present in older age groups (≥55 years: aRR 0.94, 95% CI 0.80-1.09). Lower DTG initiation in women vs men occurred early in the rollout (Dec 2019-Feb 2020: aRR 0.30, 95% CI 0.27-0.34, disappearing by Jun-Aug 2021 (aRR 0.94, 95% CI 0.87-1.03). Among people with 12-months of follow-up, attrition (aRR 0.88, 95% CI 0.81-0.97) and viraemia (aRR 0.84, 95% CI 0.77-0.93) was lower in DTG initiators. Of 177,082 adults already receiving first-line ART in Dec 2019, 122,004 (68.9%) were women and median age was 38 years (IQR 32-45). Median time on ART was 4.2 years (IQR 2.3-6.8) with the majority receiving efavirenz (98.6%) based first-line ART. By Feb 2022, 73.8% (118,253/160,171) of those remaining in care were on ART-DTG. By Feb 2022, 73.8% (118,253/160,171) of those remaining in care were on ART-DTG. Transition to ART-DTG was lower in women (adjusted hazard ratio (aHR) 0.61, 95% CI 0.61-0.62) and in pregnancy (aHR 0.50, 95% CI 0.45-0.55). Again, the effect of gender on DTG transition was only evident in younger people, and before Jun-Aug 2021.

Conclusion: In this first, large-scale analysis of first-line DTG use and outcomes in South Africa, pregnancy safety concerns likely led to young women being particularly disadvantaged, early in the rollout. As initiation with DTG was associated with better treatment outcomes, efforts to increase DTG use should be renewed.

Table 1. Part-worth utilities (zero-centered values) and relative importance scores (RIS) of LAI-ART delivery attributes and levels.

Table 2: Univariable and multivariable analysis of DTG initiation and associations with 12-month attrition and viremia, adjusted for potential confounders.
1058 MONITORING HIV DUAL-THERAPY IMPLEMENTATION AS REGIMEN SIMPLIFICATION POLICY IN BRAZIL

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Background: Long-term exposure to three-drug regimens has been associated with toxic effects such as renal and bone toxicity related to tenovifavir (TDF), cardiovascular and metabolic disorders for other ITRN. Since 2019, to minimize toxicity, Brazil recommends two-drug regimens (2-DRs) (3TC+DTG or 3TC+DRV) for those already on antiretroviral therapy (ART), with undetectable viral load (VL) and for whom the use of tenovifavir is contraindicated. Using real life data, we aimed to describe 2-DRs user profile and analyze the virological response among people living with HIV (PLHIV) in use of dual-therapy.

Methods: We obtained MoH electronic records, from 2019 to 2021, on ART prescription, HIV viral load (VL) and demographic (age, sex, geographic region and ethnicity) data. We used descriptive statistics to quantify the use of 3TC+DTG and 3TC+DRV and access VL suppression during HIV dual-therapy.

Results: From 2019 to 2021, 22,401 PLHIV used 3TC+DTG or 3TC+DRV regimens, of which 99% were ART-experienced. Of those starting 3TC+DTG, 72% was previously using DTG-containing regimens; while among those starting 3TC+DRV, 50% was taking DRV-based ART. Undetectable VL (VL < 49 copies/mL) before starting 2-DRs was observed in 96% in 2019, 97% in 2020 and 99%, in 2021. Up to 94% of those under 2-DRs were 30 years or older, 50% white/yellow, 29% black, and 58% lived in the Southeast region. Sex ratio (M/F) raised from 1.8 in 2019 to 2.0. In 2021, 3TC+DTG regimen was the preferred choice, representing 72% of first 2-DRs dispensations in 2019, and 91% in 2021. From 2019 to 2021, viral suppression raised from 92% to 97% when in use of 3TC+DTG; and 85% to 93%, for 3TC+DRV.

Conclusion: Dual-therapy regimens containing 3TC plus DTG or DRV given to ART-experienced PLHIV, with undetectable VL, provides a reasonable option for simplifying regimens, reaching satisfactory levels of viral suppression. This study further illustrates how monitoring of HIV care indicators is an effective way to validate and/or qualify health policies to guarantee proper health care for PLHIV.

1059 A NATIONWIDE OPTIMIZATION STRATEGY USING SECOND GENERATION INSTIS IN MEXICO

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Background: In Mexico until 2018, first line antiretroviral therapy (ART) was mainly based on either efavirenz-containing regimens (despite reports of high pre-treatment resistance levels); or on other regimens with drugs no longer considered optimal- protease inhibitors (PIs), nevirapine, maraviroc and first-generation integrase inhibitors (INSTIs) - all of them amenable to be switched to 2nd generation INSTIs as a single tablet regimen (STR). We aimed to evaluate a national optimization strategy through a rollout program using second-generation INSTI-based STR both for ART initiation and switch.

Methods: SALVAR (National System for Antiretroviral Surveillance and Administration) database was used for the analysis. We included adults registered in SALVAR, who started on ART or switched to BIC/TAF/FTC, from June 1st 2019 to June 30th 2021. We categorized them as “started BIC/TAF/FTC” (G1), “started other than BIC/TAF/FTC” (G2) or “switched to BIC/TAF/FTC” (G3). We calculated the proportion of participants with viral suppression (VS) (<40 copies/ml) at 6 months after ART initiation by group. We described VS and ART change by group at end of follow-up and fitted a Cox model to compare durability by group, sex and age. Durability was defined as maintaining the same ART versus changing ART regimen, LTFU or death.

Results: A total of 74,137 PLHIV were included in the analysis and followed for a median of 1.6 years (IQR: 0.8-2.1). 23,786 (31%) were classified as G1, 5352 (7%) as G2 and 44,999 (61%) as G3. Of those classified as G2: 2543 (47.5%) were started on NNRTI’s, 2021 (37.7%) on other INSTIS and 602 (11.2%) on PIs. The last regimen before switch in G3 group, was based on NNRTI’s in 67%, PI’s in 19%, other INSTIS in 10% and other regimens 4%. VS at 6 months after initiation or switch was observed in 87% individuals in G1, 80% in G2 and 98% in G3. At end of follow-up, 80% of those in G1, 56% in G2, and 87% in G3 remained with VS; <1% of individuals changed to a different regimen in G1, 4% in G2 and 1% on G3. Durability of ART regimen by group is shown in Figure 1. At initial evaluation, 31% of all individuals were on a STR regimen, vs. 96% at the end of follow-up.

Conclusion: In this real-life analysis, we observed that second generation INSTIS represent an effective and durable option for treatment optimization in the Mexican National treatment program.

Figure 1.- Durability of the same ART regimen by group.

1060 PEOPLE FAILING FIRST-LINE REGIMENS REMAIN AT RISK FOR ADVERSE SECOND-LINE OUTCOMES

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Background: People living with HIV (PLH) who have trouble maintaining adherence to 1st line regimens may develop virological failure requiring a regimen switch, but continued poor adherence remains a challenge. We assessed 2nd line treatment outcomes in the Thai National AIDS Program (NAP).

Methods: PLH aged ≥ 18 years starting 1st line (non-nucleoside reverse transcriptase inhibitor-based) ART from 2008 to 2019, who subsequently switched to 2nd line protease-inhibitor (PI)-based ART after virological failure were studied. Virological failure (VF) after 2nd line switch was defined as viral load ≥1000 copies/mL, two consecutive times after switch. Competing risks regression was used to calculate the VF cumulative incidence, and sub-distribution hazard ratios (SHR), for associations between individual characteristics and VF, with lost to follow-up (LTFU) and death as competing events. LTFU was defined as not attending clinic >12 months; mortality was confirmed by National death registry linkage.

Results: Of 299,261 people initiating ART, 29,061 (9.7%) switched to a 2nd line regimen after 1st line failure. Most (61%) were male with median age of 37 (IQR 31-44) years, median switch CD4 cell count of 149 (IQR 49-300) cells/mm3 and median 1st line ART duration of 3.6 (IQR 1.8-5.8) years. 2nd line regimens were ronivon booster lopinavir (LPV/IR) (96%), atazanavir (2%) or darunavir (DRV/c) (2%). Overall attrition after 2nd line switch was death: 4,606 (16%) and LTFU: 5,316 (18%). The cumulative incidence of VF in 25,696 PLH with viral load testing after switch was 0.85% (95% CI 0.74-0.97%), 4.13% (95% CI 3.90-4.41%), 7.96%
1061 PREDICTORS OF TRANSITIONING TO A FIRST-LINE Dolutegravir regimen in West Africa

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Background: Since 2019 Dolutegravir (DTG)-based antiretroviral therapy (ART) is recommended by the world health organization for all adults living with HIV (ALHIV) because of its tolerability and high barrier to drug resistance. Initial safety concerns in women of child bearing age have resulted in an initially lower transition in women. As most West African countries have transitioned to DTG, longer term predictors are not well documented. We describe the incidence and predictors of DTG transition (DT) in ART experienced ALHIV in West Africa.

Methods: We included all ALHIV from 6 adult cohorts in the IeDEA West Africa collaboration in Côte d'Ivoire (Cepéf, CNTS & CIRBA), Burkina Faso, Togo, and Nigeria with at least one documented visit since January 2019. Baseline follow-up was defined as the date of the DTG introduction at site-level (DISL). Patients were followed until database closure. We computed the cumulative incidence functions for DT over the follow-up period by sex in each cohort. Predictors of DT were explored using cause specific Cox proportional hazard models (with transfer, death, or loss to follow-up as competing risk). The overall follow-up time was divided into two periods (2 cox models) considering proportional hazard assumptions for sex (Early period: baseline to month 15 (P1) and late period: month 15 to database closure date (P2)).

Results: A total of 23,455 ART experienced ALHIV were included; 31% were men. Median age at DISL was 45 years (IQR: 39-52) and median follow-up was 8 years (IQR: 4-11). Overall, 14,529 (63%) DT occurred, 70% in men and 58% in women. DT transition was heterogeneous between and within countries; characterized in some cohorts (Cepéf, CNTS, NIMR) by an early steep increase in men compared to women followed by catch-up in later period among women, a persistent gap in Burkina while no difference between sex was observed in others cohorts (CIRBA, EVT-TOGO) (fig.1). Adjusted for cohort, age, ART regimen and virologic status; being a man was associated with a higher probability of DT (aHR: 2.2, 95%CI: 2.1-2.3) in P1 and a lower probability in P2 (aHR for men: 0.8, 95%CI: 0.7-0.9). In addition, DT was higher in younger (< 50 years) and virologically suppressed ALHIV during both periods.

Conclusion: In West Africa, early sex differences in DTG transition most likely related to the perinatal safety signal is gradually closing despite heterogeneity according to HIV cohorts. A continued monitoring of DT will contribute to universal and equal access to ART.

1062 Transition to Dolutegravir-based ART in Low- and Middle-income Countries in IeDEA

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Background: Since 2019 Dolutegravir (DTG)-based antiretroviral therapy (ART) for first-, second-, and third-line regimens in mid-2019 due to its tolerability and increasing levels of resistance to other ART regimens. The proportion of HIV treatment sites in low- and middle-income countries (LMICs) that have rolled out or plan to roll out DTG-based ART is unknown.

Methods: Between September 2020 and March 2021, the International epidemiology Databases to Evaluate AIDS (IeDEA) research consortium surveyed 179 HIV treatment sites in 35 LMICs to document the transition to DTG-based ART regimens and related clinical testing practices. Descriptive statistics were stratified by region.

Results: Ninety-eight percent (175) of sites completed the survey, of which 78% (137) were from 21 countries in Africa, 17% (30) from 8 countries in the Asia-Pacific, and 5% (8) from 6 countries in Latin America. DTG-based ART rollout or planned rollout by 2021 for first-, second-, and third-line regimens was reported by 95% (166), 68% (119) and 42% (73) of sites, respectively. 37% (64) of sites reported rollout or planned rollout for all three ART regimen lines; 31% (42) in Africa, 53% (16) in the Asia-Pacific, and 75% (6) in Latin America. While 97% (170) of sites reported routine viral load (VL) monitoring for patient care, 79% (139) reported that switching to DTG-based ART was based on VL testing (83% (114) in Africa, 60% (18) in the Asia-Pacific, 88% (7) in Latin America), with 70% (97) of these sites relying on VLs obtained in the prior 6 months, 20% (28) within 12 months, and 10% (14) reporting criteria that varied by patient group. 79% (139) of sites reported that HIV-1 genotypic drug resistance testing was available for routine patient care (78% (107) in Africa, 87% (26) in the Asia-Pacific, 75% (6) in Latin America), however, only 15% (26)
reported performing drug resistance testing at the time of switch to DTG-based ART (12% (16) in Africa, 30% (9) in the Asia-Pacific, 13% (1) in Latin America).

**Conclusion:** Although global HIV treatment guidelines recommend DTG-based ART for first-, second-, and third-line ART to mitigate increasing drug resistance levels, fewer than half of sites in our large global HIV consortium had or planned to fully implement these recommendations, with substantial regional variation. Incomplete rollout of DTG-based ART and suboptimal drug resistance monitoring may impede efforts to reduce HIV drug resistance, particularly in high HIV-burden settings.

Proportion of sites that have rolled out or plan to roll out dolutegravir-based antiretroviral therapy by 2021 for first-, second- and third-line ART in low- and middle-income countries in leDEA.

**1063 NATIONAL TRENDS IN ART REGIMENS FILLED FOR PEOPLE WITH HIV ON MEDICARE: 2013-2019**

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**Background:** Substantial improvements in ART regimens have occurred over the past decade, contributing to improved viral suppression and outcomes among PWH. Despite national attention on the rapidly evolving field of HIV therapeutics, empirical evidence on changes to the types of ART regimens filled by PWH over the past decade are unknown at a national level. Medicare claims data offer a unique approach to evaluate changes in prescribing patterns over time, which is increasingly important as PWH continue to age into the Medicare program.

**Methods:** Using a 20% sample of Medicare fee-for-service claims data (2013-2019), we identified PWH using the validated Medicare Chronic Condition Data Warehouse algorithm. We next used the Medicare Part D file to confirm filled ART prescriptions in each year. We identified individual ART regimens and grouped brand names with generics if they existed. We also categorized ART regimens into 1-, 2-, 3-, or 4+-tablet regimens. For PWH who switched regimens within a calendar year, we assigned them to the ART regimen that was filled for the most months during the year. We evaluated trends in the proportion of PWH who filled specific ART regimens and number of tablets in the regimen. We also identified PWH who did not fill ART prescriptions in a calendar year.

**Results:** Between 2013 to 2019, there were 36,955 unique Medicare beneficiaries with HIV in the study sample. The most common ART regimens in 2013 were EFV/FTC/TDF (15.9%), ATV+RTV+TDF/FTC (8.0%), and DRV+RTV+TDF/FTC (6.0%), which declined substantially by 2019 to 3.5%, 0.1%, and 0.4%, respectively (Figure). In 2019, the most common ART regimens among Medicare beneficiaries were INSTI-based: BIC/FTC/TAF (15.8%), ABC/DTG/3TC (11.1%), and EVG/CDBU/FTC/TAF (10.6%). The proportion of PWH on 1-tablet regimens increased from 22.1% in 2013 to 33.5% in 2019, while the proportion of PWH on 4+ tablet regimens declined from 16.1% to 3.3%. The proportion of PWH who filled no ART prescriptions declined from 7.8% (2013) to 6.0% (2019).

**Conclusion:** Among PWH in Medicare, a major shift in ART regimens occurred rapidly within a seven-year period, with the majority of Medicare beneficiaries on INSTI-based ART and 1-tablet regimens by 2019. However, more than 5% of PWH with Medicare had no ART regimens filled under Part D despite recommendations for universal ART.

**1064 NEW HIV DIAGNOSES IN THE SETTING OF PUBLICLY FUNDED PREP IN BRITISH COLUMBIA, CANADA**

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**Background:** In British Columbia (BC), Canada, antiretrovirals (ARVs) for HIV treatment and prevention are publicly funded and centrally distributed. In January 2018, tenofovir-emtricitabine based PrEP became available to eligible BC residents deemed at high risk of HIV infection. We characterize new HIV diagnoses in the first 4.5 years of the BC PrEP cohort.

**Methods:** Eligible persons enrolled in the PrEP program between 1-Jan-2018 and 30-Jun-2022 and subsequently diagnosed with HIV were included (followed to 31-Jul-2022). Client demographics, HIV risk, PrEP dispensing, HIV testing, viral load (pVL); genotypic resistance testing, and ARV treatment (ART) were described. New HIV diagnosis rate in clients dispensed PrEP at least once was calculated.

**Results:** Of 9737 clients enrolled in the PrEP program, 32 (0.3%) were diagnosed with incident HIV. All 32 were cis-men who have sex with men (MSM) with median (Q1-Q3) age 30 years (25-35), 28 (88%) resided in Greater Vancouver, 31 (97%) reported a HIV-MSM score ≥10 (median 25 [19-30]) and 31 (97%) were prescribed daily (vs. on-demand) PrEP. Median (Q1-Q3) time from enrolment to HIV diagnosis was 374 days (291-790) with a gap from prior HIV test to diagnosis of 190 days (89-314). Of 27 clients diagnosed after ≥1 PrEP dispensing, median (Q1-Q3) proportion of days covered by PrEP was 36% (14-60), with 240 days (96-326) terminal lapse in PrEP supply (based on daily dosing). The new HIV diagnosis rate in 9441 clients ever dispensed PrEP was 0.13 per 100 person-years (PY) (95% CI, 0.08-0.18) in 20,578 PY of follow-up.

Of the 32 new HIV diagnoses, 10 were acute infections, median (Q1-Q3) time to ART initiation was 7 days (4-12) with 18 starting 4-drug ART (PI INSTI, NRTI) and 14 with 3-drug ART (12 INSTI-based, 2 PI-based). Median (Q1-Q3) baseline CD4 was 600 cells/µL (432-765), fraction 32% (28-38), and pVL was >100,000 copies/mL in 53%. Mutations conferring reduced susceptibility to NRTI (n=2) or NRTI (n=7) were detected in 9 of 31 tested. All NRTI resistance was due to M184V mutations in clients with active PrEP prescription at HIV diagnosis. Of the 29 new diagnoses with follow-up >60 days, 26 (90%) achieved pVL< 40 copies/mL within 6 months.

**Conclusion:** New HIV diagnoses in a centralized, publicly funded, eligibility-based PrEP program remain low. To date, no cases of emergent PrEP-related tenofovir resistance have been observed within our cohort. Centralized HIV treatment and prevention facilitated rapid ART initiation in PrEP clients with new HIV diagnosis.
**Background:** The US Ending the HIV Epidemic (EHE) initiative has set goals to reduce national HIV incidence by 90% by 2030 and to address the disproportionate burden of HIV among Black and Hispanic/Latinx populations. The statewide HIV planning body for Florida recently released its 2022-2026 “Integrated HIV Prevention and Care Plan”, which lists strategies to increase access and uptake of HIV prevention and treatment interventions to reach the national EHE goals. Using a case study, we estimate and compare the epidemiological impact of achieving targets individually and jointly and sustaining them from 2022-2030.

**Methods:** We adapted an HIV transmission model calibrated to Miami-Dade County and adjusted the scale of HIV testing, PrEP, and ART interventions to simulate the effects of reaching targets that could be modeled from Florida’s 2022-2026 integrated plan. We defined a comparator scenario based on estimates of current population characteristics and health service access levels in Miami and set 2020 as a reference point to match the EHE timeline. Multiplicative factors were applied to reach the rate changes specified by the plan’s current and target data indicator values. Resulting increases to service access were assumed (a) proportional to existing access levels across White, Black, and Hispanic/Latinx populations, and (b) equivalently redistributed across racial/ethnic groups according to their numbers of new diagnoses in 2019. The primary outcome for each approach was HIV incidence reduction from 2022-2030.

**Results:** Compared to 2,432 infections in 2020, incidence reductions in 2030 from sustaining each of the targets in Miami would range from 1,018 (-41.8%) cases under the proportional approach to 1,423 (-58.5%) with the equivalently redistributed approach (Figure 1). The single most influential strategy was reducing new HIV diagnoses in Hispanic/Latinx men who have sex with men via increased PrEP uptake, which dropped 2030 incidence to 1,537 (-37%) cases. Achieving the current integrated care plan goals would not achieve the EHE incidence reduction target (< 243 new cases in 2030).

**Conclusion:** Achieving the goals of Florida’s current integrated care plan would reduce HIV incidence dramatically in Miami, however further efforts are required to achieve EHE Targets by 2030. Structural changes in service delivery and a focus on effective implementation of available interventions, particularly among Black and Hispanic/Latinx populations, will be crucial to overcoming the HIV epidemic in Miami, Florida.

Figure 1. Projected HIV incidence reductions, Miami-Dade County, FL: 2020 vs. 2030

**AN RCT OF HOME-BASED CARE TO REDUCE POSTHOSPITAL MORTALITY IN SOUTH AFRICA: HOMELINK**

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*Presented at CROI by a nonauthor colleague

**Background:** In sub-Saharan Africa 23% of people living with HIV (PLWH) die 6 months after discharge from a hospital. Failure to engage in care post-hospitalization is associated with mortality. We evaluated whether a series of structured post-hospitalization home visits would reduce mortality among recently discharged PLWH in South Africa.

**Methods:** We designed a home visit package with up to 6 home visits starting 1-week post-hospitalization and every 2 weeks as required thereafter. The home visit team consisted of a professional nurse and a counsellor; they used a structured assessment algorithm to evaluate participants’ social and medical needs, obtained direction from a doctor for further guidance, collected specimens for laboratory testing, or referred the participant for further evaluation. We compared this intervention to care as usual in a pilot randomized trial conducted at a single hospital in South Africa. The primary goals of the study were to determine feasibility and acceptability and identify opportunities to improve the home visit intervention. We report effectiveness for PLWH based on the primary outcome of all-cause mortality 6 months after discharge from hospital.

**Results:** We enrolled 125 PLWH who were randomized 1:1 to home visit intervention or care as usual; 14 were late exclusions because they died prior to discharge (n=13) or had a prolonged hospital stay (n=1). In the 111 PLWH included in the analysis, the median (interquartile range [IQR]) age was 39 (33, 48) years, 69% were women, the median duration of the index hospitalization was 7 (3, 12) days, and primary reasons for the index hospitalization included TB (31%), heart and/or lung related diseases (22%), non-TB/COVID infections (25%), and anemia (15%). Most [96% (n=115/126)] intervention arm participants received ≥ 1 home visits. By six months 14 (13%) participants died: 4 (7%) in home visit intervention arm and 10 (18%) in the care as usual arm (p=0.09). A similar proportion of readmissions occurred by arm: 20 (36%) in the home visit arm and 22 (39%) in care as usual.

**Conclusion:** Home visits done after discharge from hospital provided care to an extremely vulnerable group of PLWH at very high mortality risk. We demonstrated both feasibility and preliminary efficacy of delivering post-hospital visits. Structured home visits appear to be a promising approach that would benefit PLWH. Larger studies in diverse populations with cost-effectiveness components are required.
analysis. We then conducted ad-hoc analyses to compare interventions from IDEaL to SOC from ENGAGE. In-depth interviews were conducted with a subset of men.

Results: 1149 MLHIV out of treatment were enrolled in IDEaL (n=515) and ENGAGE (n=634) between August 2021-September 2022. In ENGAGE, (re-)initiation was significantly higher in the MC+Home ART (3-month) vs SOC arm (RR: 1.28 [95%CI 1.19-1.36]). In IDEaL, (re-)initiation was equally high across all intervention arms with no significant differences (P=98%). In ad-hoc analyses, compared to ENGAGE SOC, (re-)initiation was significantly higher across all IDEaL intervention arms: MC+Facility ART (RR: 1.28 [95%CI 1.16-1.42]); MC+Home ART (1-month) (RR: 1.30 [95%CI 1.14-1.42]); and Stepped intervention (RR: 3.81 [95%CI 1.03-1.88]) (Table). All interventions had high satisfaction (96%) and low unwanted disclosure (~1%). Men in interviews (N=45) valued being active participants in HIV services and desired ongoing relationships with healthcare workers. To promote retention men requested longer drug dispensing and increased privacy at facilities.

Conclusion: Male-specific counseling alone and combined with home-based ART improved men's ART (re-)initiation in Malawi. Longer term outcomes will indicate if ART gains will be sustained.

HIV outcomes for MLHIV with treatment interruption across interventions in Malawi: reported by MLHIV (n=1149)
Table 1. Factorial analysis of risk factors for HIV status awareness, linkage to ART and viral suppression following HITS visit among HIV positive men and women, 2018-2019 (n=9,989).

<table>
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<th>Women (n=1,813)</th>
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**Results:**
- Between January 2010 and December 2020, 15,371 individuals attended at least one clinic visit: 188 (1%) transgender women and 15,183 (99%) MSM.
- Over time, the majority of transgender women and MSM were retained in care, received ART and were virally suppressed (Figure), albeit with greater gaps for transgender women than MSM. Of 170 transgender women and 13,532 MSM linked to care in 2020, fewer transgender women than MSM were retained in care (89% vs 95%, p=0.004, used ART (88% vs. 94%, p<0.001) and were virally suppressed (84% vs. 92%, p<0.001). The proportion of transgender women newly diagnosed with HIV ranged from 6% (4/67) in 2010 to 6% (9/156) in 2020; for MSM, this varied from 8% (699/8,756) in 2010 to 2% (215/12,892) in 2020. The proportion of transgender women who were late presenters varied between 25% and 75% over time, while for MSM it varied between 37% and 46%.

**Conclusion:**
- Over a 10 year time period, the vast majority of transgender women and MSM diagnosed with HIV in the Netherlands were linked to and retained in care, received ART and were virally suppressed. The HIV care continuum for transgender women continues to lag behind across its stages and late presentation remains more common. Identifying barriers to HIV care and designing targeted interventions, jointly with the transgender community, will be crucial to improve HIV care retention and outcomes.

**1070 THE HIV CARE CONTINUUM AMONG TRANSGENDER WOMEN IN 2010-2020 IN THE NETHERLANDS**

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**ATHENA observational HIV cohort**

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**Background:**
Transgender women are at increased risk for acquiring HIV and earlier studies observed low retention in HIV care, as well as lower rates of antiretroviral therapy (ART) uptake, adherence and viral suppression. We compared HIV care retention between transgender women and men who have sex with men (MSM) in the Netherlands. Additionally, we compared the proportion of new HIV diagnoses those in care per year and proportion of late presenters.

**Methods:**
Using data from the ATHENA cohort and a repeated cross-sectional design, we assessed the different stages of the HIV care continuum (linkage to care, retention in care, ART use, and viral suppression) among transgender women and MSM between 2010 and 2020. We described new HIV diagnoses among all individuals living with HIV within a calendar year. The proportion of individuals with a late diagnosis was calculated by dividing the number of late presenters (defined as a CD4 count of < 350 cells/µl or an AIDS-defining event) by the number of newly diagnosed individuals in a given year.

**Results:**
- Between 2010 and 2020, 20% of MSM who acquired HIV were late presenters (defined as a CD4 count of < 350 cells/µl or an AIDS-defining event), compared to 46% of transgender women. The proportion of MSM linked to care increased from 25% (699/2,823) in 2010 to 75% (9,989/13,249) in 2020, while for transgender women this varied from 8% (4/67) in 2010 to 75% (9/156) in 2020. Over time, the majority of MSM and transgender women were retained in care (89% vs. 95%, p=0.004), used ART (88% vs. 94%, p<0.001) and were virally suppressed (84% vs. 92%, p<0.001). A higher proportion of transgender women newly diagnosed with HIV ranged from 6% (4/67) in 2010 to 6% (9/156) in 2020; for MSM, this varied from 8% (699/8,756) in 2010 to 2% (215/12,892) in 2020. The proportion of transgender women who were late presenters varied between 25% and 75% over time, while for MSM it varied between 37% and 46%.

**Conclusion:**
- Over a 10 year time period, the vast majority of transgender women and MSM diagnosed with HIV in the Netherlands were linked to and retained in care, received ART and were virally suppressed. The HIV care continuum for transgender women continues to lag behind across its stages and late presentation remains more common. Identifying barriers to HIV care and designing targeted interventions, jointly with the transgender community, will be crucial to improve HIV care retention and outcomes.

**1071 PROJECTED IMPACT OF IMPROVING HIV CARE ON LIFE EXPECTANCY AMONG BLACK AND WHITE MSM**

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**Background:**
Inequities persist between non-Hispanic Black (BMSM) and White (WMSM) MSM with HIV. Rates of engagement in care, and viral suppression (VS) vary by race-stratified life tables for increased tobacco-related mortality, given the high prevalence of tobacco use among MSM with HIV. We conducted sensitivity analysis by varying HIV testing frequencies (6m; annually; 6y), engagement in care and VS for Black MSM (BMSM) and White MSM (WMSM) with HIV.

**Methods:**
Using the validated CEPAC microsimulation HIV model, we projected LE among BMSM and WMSM who acquire HIV under simulated Status Quo HIV care conditions. Status Quo HIV care was modeled using race-stratified 2019 data from the Centers for Disease Control and Prevention and estimates of: 1) average age at HIV infection (BMSM: 26.8y, WMSM: 35.6y), 2) average HIV testing frequency (BMSM: every 4.9y, WMSM: every 4.1y), 3) percent of time engaged in care for the 5y after diagnosis (BMSM: 75.2%, WMSM: 80.6%), and 4) percent of time with VS while in care for ART initiation (BMSM: 82.0%, WMSM: 91.2%). To account for non-HIV-related mortality, we adjusted national race-stratified life tables for increased tobacco-related mortality, given the high prevalence of tobacco use among MSM with HIV. We projected LE to be 67.2y (BMSM) and 73.5y (WMSM) for BMSM and 38.3y for WMSM. We projected LE to be 67.2y (BMSM) and 73.5y (WMSM) with Status Quo care. In sensitivity analysis (Figure 1), BMSM would gain 0.7 or 0.9 life years (LY) if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS).

**Results:**
Among MSM who acquire HIV, average age at diagnosis was 30.3y for BMSM and 38.3y for WMSM. We projected LE to be 67.2y (BMSM) and 73.5y (WMSM) with Status Quo care. In sensitivity analysis (Figure 1), BMSM would gain 0.7 or 0.9 life years (LY) if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS); WMSM would gain 0.7 or 0.9LY if testing frequency improved to annually or every 6 months, respectively (assuming Status Quo engagement in care and VS).

**Conclusion:**
Projected LE for Black MSM with HIV is substantially lower than white MSM with HIV, but inequities decline with improved HIV testing, engagement in HIV care, and VS for Black MSM. Our findings highlight the need for an equity-driven approach to HIV care interventions around testing and engagement in care.
1071.5 RETENTION IN CARE AMONG PERUVIAN MSM AND TGW IN A REAL-WORLD HIV PrEP PROGRAM

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Background: Tenofovir-based daily oral HIV pre-exposure prophylaxis (HIV PrEP) is an effective HIV prevention modality, but sustained use over time is needed for continued protection among individuals at high risk for HIV exposure. Suboptimal adherence and retention in care threaten to diminish the impact of HIV PrEP on reducing HIV burden. PrEP PERU is an ongoing, multi-site, prospective cohort study evaluating HIV PrEP implementation among adult men who have sex with men (MSM) and transgender women (TGW) accessing care at non-government health centers in Peru. We sought to evaluate HIV PrEP adherence and retention in care among PrEP PERU participants prior to the onset of COVID-19 service disruptions.

Methods: We analyzed baseline and follow-up data from the PrEP PERU study through 3/15/2020, the first day of Peru’s COVID-19 lockdown. MSM and TGW ≥18 years of age with at least one HIV risk factor were eligible for enrollment. After the first follow-up visit at 4 weeks, TDF/FTC refills and clinic visits occur quarterly, at the discretion of the prescribing clinician. The medication is provided free of charge, but participants pay for laboratory testing plus a small service fee for clinic visits. Data is collected at baseline and quarterly follow-up visits on sexual risk behaviors and HIV PrEP use. We used bivariate analysis to evaluate the association between baseline factors and 6-month HIV PrEP retention in care. As a proxy for adherence, pharmacy dispensation records were used to calculate the proportion of days covered (PDC) by TDF/FTC.

Results: Overall, 351 participants started TDF/FTC at four study sites in Lima from 1/23/2017 to 3/15/2020. Of this analysis population, 94% were cisgender men, 10% identified as bisexual, and median age was 31 (interquartile range [IQR], 27 – 38). Among those with at least 6 months of observation time (n=302), 91% attended ≥1 follow-up visit and 77% attended ≥2 follow-up visits during the 6 months after enrollment. The proportion with favorable adherence (PDC ≥0.8) was 85%. There were 6 confirmed HIV seroconversions in the analysis period (1.2 per 100 person-years).

Conclusions: In this analysis of HIV PrEP outcomes among MSM and TGW prior to COVID-19 pandemic disruptions in Peru, over 3/4 of the population remained in care and had favorable measures of adherence during the first 6 months after starting HIV PrEP. This level of HIV PrEP engagement compares favorably to reports from similar settings of HIV PrEP implementation in Latin America.

1072 PrEP KNOWLEDGE, EVER-USE, AND DISCONTINUATION AMONG LAKE VICTORIA FISHERMEN IN UGANDA

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Background: There is limited population-level data on the PrEP care continuum in eastern Africa. We assessed PrEP knowledge, ever-use, and discontinuation at the population-level following PrEP rollout in a Lake Victoria fishing community in Uganda with 40% HIV prevalence.

Methods: We used cross-sectional data collected between June 2018 and November 2020 from a Lake Victoria fishing community under surveillance in the Rakai Community Cohort Study (RCCS) to measure levels of self-reported PrEP knowledge, ever-use, and discontinuation following PrEP rollout in key populations in 2017. Our analysis included HIV-negative persons who reported having ever received an HIV test result, with discontinuation measured among those reporting ever-use only. We also examined associations between demographic, behavioral, and health utilization factors with each outcome using modified Poisson regression adjusted for age with all analyses stratified by gender. Associations were reported as adjusted prevalence ratios (adjPR) with 95% confidence intervals (95%CI).

Results: There were 1,401 HIV-negative participants, of whom 1,363 (97%) reported ever receiving an HIV test result. Median age was 29 years (IQR: 23-36), and 42% (n=577/1363) were women. While PrEP knowledge was high (85.5%), PrEP ever-use was low (14.5%), with no significant differences in levels of knowledge or ever-use by gender. Among those likely PrEP eligible as assessed from RCCS demographics and self-reported risk behavior (n=514), PrEP ever-use was 22.4%. Having ever used PrEP was strongly associated with perceived HIV risk: those reporting higher perceived risk were more likely to report PrEP ever-use compared to those who reporting not being at risk (p<0.001). Women reporting transactional sex also were more likely to report PrEP use vs. women who did not (adjPR=1.81; 95%CI: 1.21-2.71), as were those reporting a recent HIV test in the last year vs. those who did not (men: adjPR=2.98; 95%CI:1.58-5.62; women: adjPR=4.03; 95%CI:1.80-9.01). Among 116 men and 81 women who reported ever using PrEP, 48.3% and 46.9% discontinued PrEP, respectively.

Conclusion: In this community with high HIV prevalence, there were low levels of self-reported PrEP use and high rates of discontinuation despite high levels of PrEP awareness. Efforts that enhance awareness of HIV risk and increase access
to PrEP through HIV testing may help increase PrEP use among HIV-negative persons in African settings with high HIV burden.

1073 STRUCTURAL INFLUENCES ON PREP CASCADE AMONG YOUNG WOMEN IN POST-ABORTAL CARE IN KENYA

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Background: Women accessing care at post abortion care (PAC) clinics have had recent and potentially ongoing condomless sex, placing them at risk for subsequent unintended pregnancy, HIV, and other STIs depending on their geographic settings. Few studies have assessed PAC settings for delivery of HIV prevention services, including pre-exposure prophylaxis (PrEP), and for influential structural factors.

Methods: Using medical records data abstracted from adolescent girls and young women (AGYW) aged 15 to 30, we describe PrEP offers, uptake, and refills within an implementation science project that launched PrEP delivery in 9 private and 6 public PAC clinics in Kenya. Poisson regression models were used to estimate the effect of clinic- and provider-level factors, determined by technical assistants, on PrEP outcomes.

Results: From March to August 2022, 1945 AGYW in PAC settings were offered PrEP, of which 403 (20.7%) initiated PrEP and 30 (7.4%) of those received at least one refill. Among PAC clinics, PrEP offers were more common among clinics that had fully incorporated PrEP into routine clinical tasks (vs. separate, prevalence ratio (PR): 1.17 95% CI: 1.03, 1.33) and less common in clinics that were private (vs. public, (PR): 0.84, 95% CI: 0.77, 0.92), had low client volume (PR: 0.5, 95% CI: 0.45, 0.54), had sufficient staffing (PR: 0.14, 95% CI: 0.11, 0.18) or space (PR: 0.84, 95% CI: 0.77, 0.92), and lacked a champion PrEP provider (PR: 0.86, 95% CI: 0.78, 0.94). The frequency of PrEP uptake was higher among clinics with sufficient space (PR: 1.53, 95% CI: 1.26, 1.87) and lower among clinics that were private (vs. public, PR: 0.4, 95% CI: 0.33, 0.49), had a low client volume (PR: 0.33, 95% CI: 0.27, 0.41), had sufficient staffing (PR: 0.34, 95% CI: 0.08, 0.25), and lacked a champion PrEP provider (PR: 0.74, 95% CI: 0.6, 0.9). PrEP refills were less frequent among clinics that had at the same point of care for refills (vs. different, PR: 0.05, 95% CI: 0.02, 0.12), were private (vs. public, PR: 0.05, 95% CI: 0.01, 0.22), and had a low client volume (PR: 0.15, 95% CI: 0.06, 0.39).

Conclusion: PrEP outcomes for AGYW accessing services integrated into PAC are influenced by larger structural factors in the healthcare ecosystem, including clinic structure, staffing, and the presence of PrEP champions. Young women accessing PrEP services may benefit from the sufficient space and privacy in PrEP program clinics and access to PrEP champions.

1074 HIGH LEVEL OF HIV PREVENTION-EFFECTIVE CONTINUATION IN A LARGE PREP PROGRAM IN KENYA

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Background: HIV prevention-continuation (i.e., prevention-effective use) is unknown for most PrEP programs. Two-thirds reported it was their own decision to start PrEP and a third a shared client-provider decision. Overall, 73% reported their HIV risk status changed (feeling no longer at risk, U=U with virally suppressed partner, or separation from partner) and that was the primary reason for PrEP stop. Pill burden (9%) and side effects (11%) were relatively uncommon primary reasons for PrEP stop. Notably, >86% were satisfied with experience at the last clinic visit; <1% attributed stopping to clinic factors. Overall, no method at all (24%), not feeling at risk (39%), practicing U=U (19%), no sexual partner (26%), condom (14%) were common HIV prevention choices practiced at time of the interview. Importantly, the majority (94%) were satisfied with their current HIV prevention method choice.

Conclusion: Nearly three-quarters of PrEP discontinuations in large national public PrEP program in Kenya were appropriate PrEP non-use aligned with low HIV risk states or other prevention strategies, and almost all clients were satisfied with their current HIV prevention choice. Our findings illustrate that using client-level PrEP continuation rates without contextual dynamic individual risk and use of other HIV prevention options is not an appropriate measure of real-world PrEP program success.

1075 DEPRESSION ASSOCIATED WITH PREP HOLIDAYS AMONG KEY POPULATIONS IN NAMIBIA

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Background: Mental health challenges negatively impact HIV outcomes, including engagement in prevention services. Key populations may be more vulnerable to these impacts, particularly in the context of oral PrEP, which requires frequent dosing decisions and ongoing engagement with the health system. Mental health among oral PrEP users in Africa has not been well described.

Methods: We enrolled new and continuing PrEP users from 13 facilities in Namibia in a cross-sectional study characterizing PrEP use decisions and determinants among adolescent girls and young women (AGYW) and key populations. We measured mild, moderate and acute depression using the Patient Health Questionnaire (PHQ9). A referral pathway was established to link clients with acute depression or suicidal ideation to mental health services. We also measured self-reported alcohol and drug use, HIV risk perception, condom use, “PrEP holidays,” missed pills, and side effects. We described relationships between the variables with chi-square tests and used log binomial regression to estimate mental health impacts on PrEP holidays. The study was approved by the JHU and Namibia MOHSS IRBs.

Results: The study (n=500) included 39 MSM, 28 male sex workers, 254 AGYW, and 214 FSW. Mean age was 25.5 (SD = 6.6). Overall, 222 (44%) vs. 278 (66%) were new vs. continuing PrEP users. Among 499 providing responses to the PHQ9, 11% and 5% had scores suggesting moderate and acute depression, respectively; 29% reported suicidal ideation. FSW were more likely to report acute depression (p<0.001). In unadjusted analyses, taking a “PrEP holiday” was associated with depression (p=0.03), alcohol use disorder (p<0.01), and drug use (p<0.01). Depression was further associated with missing pills on weekends (p=0.02), reporting side effects (p=0.04), HIV risk perception (p<0.001), and drug use among FSW (p=0.001) and MSM (p=0.02). In adjusted analyses, depression predicted PrEP holidays among MSM (B=3.4; SE: 1.7).

Conclusion: Clients accessing oral PrEP represent marginalized populations who may be at greater risk for mental health issues. PrEP can be an entry point for screening and referral to mental health services. Programs should integrate mental health with HIV services, to address high rates of depression and support continuation in HIV services.
1077 A MODEL OF SUCCESSFUL ART INITIATION IN THE CONTEXT OF MASSIVE CIVIL UNEASY IN HAITI

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Background: Haiti is experiencing massive civil unrest and violence, with armed gangs in control of over 50% of the country. This has major consequences for HIV care, as kidnappings are common, and roads are frequently blocked due to protests or gang-related activity; many Haitian health care providers have left the country.

Methods: The Gheskio Centers, in Port-au-Prince, Haiti, is located at the epicenter of gang activity. They have developed a model of care for initiating tenofovir-lamivudine-dolutegravir (TLD) in the midst of major civil unrest which includes: (1) Staff have created a welcoming environment, based on dignity and respect for everyone; (2) Patients are called in advance of each visit, and on day of a missed visit; patients who cannot be reached by phone are visited at home; (3) Patients who can’t come to the Gheskio clinic are offered ART at community ART refill sites; ART is provided at home for patients who can’t leave their home medication delivery) when it is too dangerous to travel to the main Gheskio facility; (4) Hot meal at every study visit, plus a package of staple foods for home; and (6) Transportation subsidy and phone card at each visit (combined cost of $2.00/visit). As an example of the impact these services have on research, we report retention and viral suppression rates for an ongoing trial of participants with viral suppression on second-line ART; participants were randomized to continue a boosted protease inhibitor regimen or switch to bictegravir/tenofovir alafenamide/emtricitabine. We report the outcomes for participants with 48 weeks of potential follow-up time for both groups combined.

Results: From October 30, 2020, to September 6, 2021, 208 participants were enrolled in the study. 115 (56%) were female, with a median age of 35 (IQR: 22, 57); all had HIV-1 RNA < 200 copies/mL at screening. Of these, 198 (95.2%) had HIV-1 RNA < 200 copies/mL during the 48-week visit window; 1 (< 1%) had HIV-1 RNA >200 copies/mL during the 48-week visit window; 1 (< 1%) was lost to follow-up with HIV-1 RNA >200 copies/mL; and 8 (3.8%) had HIV-1 RNA < 200 copies/mL at last visit but no viral load during the 48-week visit window. 2 died (1 from gun violence and 1 died of metastatic cervical cancer), 13 (6.7%) persons were lost to follow-up, and 13 (6.7%) remain in care without 12-month viral load testing. Of the 77 (72.6%) who received 12-month HIV-1 RNA testing, 71 (92.2%) had < 200 copies/mL.

Conclusion: Outstanding research outcomes are possible in settings of severe political and civil unrest, with a comprehensive approach to social and nutritional support for study participants.
1079 8-YEAR CARE TRAJECTORIES IN AN URBAN COHORT OF PWH RECEIVING CARE: WASHINGTON, DC
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Background: As many as 50% of PWH are not engaged in care and others cycle in and out of care. We sought to identify and characterize different groups of longitudinal care trajectories among a cohort of PWH who have been linked to HIV care in Washington, DC.

Methods: We used data from participants in the DC Cohort, a longitudinal multi-site cohort of PWH receiving care at 14 clinics in DC, who were ≥18 years and enrolled from 1/1/2011 to 6/30/2021. To identify longitudinal care trajectory groups we used grouped-trajectory modelling. Participants were considered “engaged” if they had ≥1 HIV visit, CD4 or VL during a specified 200-day interval. Time-stable risk factors were added to the group membership probabilities to identify predictors of class membership using multinomial logistic regression. Time-varying risk factors of viral suppression (VS [HIV RNA < 200 copies/ml]) and the modified Quan-Charlson comorbidity index (QCCI), a predictor of mortality, were added to the model of the trajectory shapes.

Results: 11,064 participants were included in the analysis (baseline: median age 47.3 years, 72% male, 63% non-Hispanic Black, 48% ≥10 years since HIV diagnosis, 75% VS). Four latent trajectory groups were identified: those with high engagement (45%), medium engagement (26%), gradual disengagement (13%), and early disengagement (15%). Compared to those with high engagement, those with early disengagement were significantly more likely at enrollment to have a CD4<500 cells/µL, not be VS, homeless, privately insured, younger, diagnosed within 4 years, and ARV naive (all p < 0.05). Those with gradual disengagement were significantly more likely to not be VS, younger, male, and diagnosed within 4 years compared to those with high engagement (all p < 0.05). Those with medium engagement were significantly more likely to have CD4<500 cells/µL, not be VS, privately insured, and younger compared to those with high engagement (all p < 0.05). When including time-varying covariates of VS and co-morbidity, not being VS significantly lowered the probability of engagement whereas the probability of engagement significantly increased with increasing numbers of co-morbidities using the QCCI (Figure). Conclusion: Identifying characteristics of those disengaged in care using longitudinal approaches can help guide intervention development to improve HIV care engagement. To further promote optimal long-term care engagement, differentiated care models may be needed at various stages of a PWH’s HIV care trajectory.

Figure: Estimated trajectory groups for the main model (A), time-varying viral suppression (B), and modified Quan Charlson Co-morbidity Index (QCCI) (C).

1080 PREDICTORS OF RETENTION IN THE WOMEN’S INTERAGENCY HIV STUDY: 2011-2019
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Background: Women of color and women of low socioeconomic status (SES) are underrepresented in HIV research, but their participation is crucial to improving HIV outcomes. We assessed baseline factors associated with visit attendance and dropout in women with HIV (WWH) and women without HIV in the Women’s Interagency HIV Study (WHIS).

Methods: Women enrolled in WHIS from 2011-2015 were followed for up to 10 consecutive semiannual visits through 2019. Dropout was defined as no further attended visits with no passive follow-up and measured from the first missed visit. We assessed the association of baseline factors with full (100%), partial (71%-99%) and low (<70%) visit attendance rates by chi-square and then performed multivariable ordinal logistic regression adjusting for HIV status and factors with p < 0.10 in the bivariate analysis. A separate model in WWH included HIV-related factors. We then identified factors associated with early (visits 2-3), intermediate (visits 4-6) and late (visits 7-10) dropout compared with no dropout by chi-square.

Results: 1214 (886 WWH, median age 44 years, 81% Black, 69% from study sites in the U.S. South, 59% income < $12,000/year) participants were included. 76% had full visit attendance, 14% had partial visit attendance, and 10% had low visit attendance. In the adjusted model, greater visit attendance was associated with age >50, Black race, living in one’s own residence, study site in the U.S. South, and history of hypertension (Table). Among WWH, greater visit attendance was associated with age >50, Black race, taking antiretroviral therapy (ART), and study site in the U.S. South. The retention rate was 81%. Early, intermediate, and late dropout occurred in 65.5%, 78.7%, and 82.8% participants, respectively. Compared with no dropout, early dropout was associated with more depressive symptoms (CES-D score > 16), lower health perception, smoking, HIV RNA > 200 copies/ml, not on ART, and CD4 count < 200 cells/mm3. Intermediate dropout was associated with non-Black race, no history of diabetes, and being born outside the U.S. Late dropout was associated with employment, smoking, HIV RNA > 200 copies/ml, not on ART, ever experiencing physical violence, and not getting regular HIV care.

Conclusion: Retention in WHIS of predominantly Black, low SES women with and without HIV was excellent. Different baseline factors were associated with different study dropout times suggesting that different retention strategies may be required over time in a longitudinal HIV cohort study.

Table. Bivariate and multivariable association of participant characteristics at study enrollment with WHIS visit attendance
1081 RETENTION IN OPIOID AGONIST THERAPY AMONG PEOPLE LIVING WITH HIV IN BRITISH COLUMBIA
Kiana Yazdani, Kate Salters, Katerina Dolguihik, Monica Ye, Jason Trigg, Ronald Joe, David Moore, Julio Montaner, Rolando Barrios *
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*Presented at CBNA by a non-author colleague

Background: The illicit drug toxicity crisis in British Columbia (BC) has reduced the survival gains among people living with HIV (PLWH) achieved by combination antiretroviral therapy. Supporting PLWH with concurrent opioid use disorder (OUD) to engage and remain on opioid agonist therapy (OAT) is necessary to optimize HIV care. We examined correlates of retention on OAT among a cohort of PLWH who received at least one OAT dispensation in BC.

Methods: We analyzed data from the Seek and Treat for Optimal Prevention of HIV/AIDS database between April 1996 and March 2017. Those with known gender, age of ≥19 years old, and ≥12 months of follow-up were included. We identified OAT dispensation as the receipt of methadone or buprenorphine/ naloxone (introduced to BC in 2008) through the PharmaNet database. Treatment episodes with no interruptions in the prescribed dosages lasting ≥3 days for methadone, or ≥6 days for buprenorphine were constructed. A period of continuous retention in treatment was evaluated as no interruption in the prescribed dosages for at least 12 months. We examined temporal trends in retention over the calendar years. A generalized estimating equation (GEE) model was built to assess correlates of 12-month retention in OAT. Of note, in the present setting, methadone was the recommended treatment in BC until July 2017.

Results: A total of 13,433 PLWH were included in the analysis of whom 2,151 (16.01%) had at least one OAT dispensation (methadone: 2,075; buprenorphine: 76). Median (Q1, Q3) age was 37 years (31, 43) and 60.1% (n=1,293) were male. PLWH initiated on buprenorphine versus (vs.) methadone were more likely to be older 42.5 (34, 52) vs. 37 (31, 43), and had a significantly higher comorbidities n (%): depression 40 (52.6) vs. 821 (38.6); chronic pain 39 (51.3) vs. 440 (21.2), hepatitis C 47 (61.8) vs. 802 (38.6); chronic obstructive pulmonary diseases 6 (7.80) vs. 65 (3.3); cancer 22 (28.0) vs. 254 (12.2). There was a decline in retention at the rate of 1.34% per year (p<0.0001). Table 1 presents correlates of retention among PLWH.

Conclusion: Our findings indicated that OAT retention among PLWH has declined over time. Higher odds of retention were associated with a ten-year increase in age, previous retention history, achieving therapeutic dose (≥ 60mg methadone; ≥12mg buprenorphine), and methadone treatment. To meet and sustain the UN 95-95-95 by 2025 Targets among PLWH with comorbid OUD, optimal management of OUD and implementation of OAT aimed at maximized retention is necessary.

Table 1. Correlates of retention in OAT among PLWH between April 1996-March 2017

<table>
<thead>
<tr>
<th>Variable</th>
<th>Retained (n=2,151)</th>
<th>Not Retained (n=11,282)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&gt;30 yrs)</td>
<td>241 (11.2)</td>
<td>1,910 (16.8)</td>
<td>1.00 (95% CI)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>1,293 (60.1)</td>
<td>1,496 (13.8)</td>
</tr>
<tr>
<td>Methadone</td>
<td>Yes</td>
<td>1,671 (76.6)</td>
<td>494 (4.4)</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Yes</td>
<td>480 (22.4)</td>
<td>10,788 (95.6)</td>
</tr>
</tbody>
</table>

1082 A RANDOMIZED CONTROL TRIAL OF AN HIV CARE INTERVENTION BUNDLE FOR PEOPLE WITH HIV
Joseph D. Perazzo, Tamilyn Bakas, Gutaiba Oudat, Joshua Lambert, Carl J. Fichtenbaum
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Background: Prompt initiation of antiretroviral therapy (ART) is now considered the standard of HIV care. Factors associated with delayed or inconsistent HIV care HIV care include: poverty, lack of transportation, lack of healthcare coverage and fear of HIV stigma. Strengths-based case management, intensive outreach, and patient navigation are well-supported interventions to improve care that have largely been tested/implemented in isolation. We developed an HIV care intervention bundle that combines these strategies using pre-/post-visit telehealth calls to provide psychosocial and educational support, health system navigation, and enhanced healthcare communication.

Methods: We conducted a longitudinal randomized control study to compare participant time to viral suppression and care-related measures (eg visit adherence). Participants were randomized to receive the intervention (n=20) or receive the standard of care (n=20). Intervention participants received up to seven additional telehealth sessions with a Registered Nurse. Intervention participants were screened for psychosocial needs, addiction services, clinical symptoms, and referred for assistance when necessary. One-on-one education and psychosocial support were provided to the intervention group. Virologic and care-related data were abstracted through prospective record review. Participation lasted one year with measures evaluated at baseline, six-months, and 12-months.

Results: Our sample included 40 adult men (70%) and women (30%) with detectable viral loads (>1000 copies), aged 18-65 (μ=36.30±10.82 years) who were African American (50%), White (47.5%), and multi-racial (2.5%). There were no significant differences in the distribution of gender, age, race, or baseline viral load or antiretroviral uptake between the intervention and control groups. Intervention participants reached viral suppression significantly more quickly than control participants (see table, p<0.04), were more likely to complete scheduled visits (p<.005), were less likely to be lost to follow-up in the first year (p<.04), and to be undetectable at the 12-month time point (p<.01)

Conclusion: Our results demonstrate the potential of a telehealth intervention bundle to help patients reach viral suppression sooner, promote early identification of distress and difficulties, and help strengthen clinical relationships with the healthcare team. A healthcare bundle which focuses on supporting the needs of PWI may improve overall health and reduce the spread of HIV.

Primary and Secondary Outcomes

1083 LONG TERM OUTCOMES OF RAPID ANTIRETROVIRAL THERAPY IN AN INTEGRATED HEALTH SYSTEM
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Kaiser Permanente Northern California, Oakland, CA, USA

Background: In 2017, the World Health Organization recommended rapid ART based on studies showing improved virologic suppression and care retention. Prior studies on rapid ART examined short term outcomes (1-2 years follow-up) with small cohort sizes. Here, we compare both short- and longer-term clinical outcomes of patients newly diagnosed with HIV who received rapid or standard ART strategies.

Methods: This is an observational cohort study of adults ≥18 years old newly diagnosed with HIV between January 2015 - December 2020 who initiated ART within one year of diagnosis at Kaiser Permanente Northern California (KPNC). Rapid and standard ART were defined as initiation ≤7 days versus >7 days after HIV diagnosis, respectively. Using electronic health records, data
were collected on HIV viral load (VL), clinical encounters, and ART refills. Short term outcomes included time to virological suppression (< 200 copies/ml) and retention in care at one year after diagnosis. Retention in care was defined as ≥1 VL measurement and 2 HIV primary care office visits at least 3 months apart within 1 year of diagnosis. Long-term outcomes included viremia copy-years (VCY), defined as the area under patients’ longitudinal VL curve, and proportion of days covered (PDC) with dispensed ART until lost to follow up or 31 December 2021. Differences between groups were assessed using Chi-Square and Kruskall Wallll tests for categorical and continuous data, respectively.

**Results:** Median days to viral suppression was shorter in the rapid ART group (48 vs 77, p < 0.001). At one year from diagnosis, no significant difference in virologic suppression was found between groups, and the standard ART group had higher retention in care (81.5% vs 76.3%, p < 0.05). Over the study duration, however, patients in the rapid ART group had improved median PDC (100% vs 90%, p < 0.001) and lower median VCY (3.6 vs 3.8 log_{10} copy x year/mL, p < 0.01).

**Conclusion:** While one-year follow-up showed similar levels of viral suppression and improved retention in care with standard ART, over a longer period of follow-up, individuals with rapid ART had higher PDC and lower VCY. These findings suggest that rapid ART may improve long-term clinical outcomes compared to standard ART. Lower retention in care in the rapid ART group highlights opportunities for better engagement in care soon after rapid ART initiation.

Outcomes of KPNC adults newly diagnosed with HIV between 2015-2020

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall</th>
<th>Standard ART</th>
<th>Rapid ART</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median days toVL &lt;500 copies (NRF)</td>
<td>60 (50–100)</td>
<td>71.8 (54–120)</td>
<td>64.0 (51–90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VL &lt;200 copies within one year of HIV diagnosis</td>
<td>1.29 (1.0–1.4)</td>
<td>0.80 (0.6–1.0)</td>
<td>0.63 (0.4–0.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Retained in care one year after diagnosis</td>
<td>1,125 (78.7)</td>
<td>775 (81.2)</td>
<td>348 (78.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Median proportion of days covered with ART (PDC)</td>
<td>1.0 (0.0–1.0)</td>
<td>0.5 (0.0–1.0)</td>
<td>1.0 (0.0–1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median virologic suppression (log_{10} copies/mL)</td>
<td>5.8 (5.1–4.4)</td>
<td>5.8 (5.1–4.4)</td>
<td>5.0 (4.3–4.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Background:** The HIV care continuum emphasizes that PWH should engage in lifelong care and treatment to achieve viral suppression (YS), yet progress along the continuum is non-linear for many PWH. We sought to characterize states of engagement in care and identify potential factors associated with disengagement among a longitudinal cohort of PWH receiving care at 14 HIV clinics in Washington DC enrolled in the DC Cohort.

**Methods:** DC Cohort participants ≥18 years who enrolled from 1/1/2011 to 6/30/2021 were included. Longitudinal care engagement was determined using discrete multistate modelling using 6 mutually exclusive states (engaged, short, medium-, long-term disengaged, transferred, died) based on care engagement (i.e., having ≥1 HIV visit, CD4 or VL in a 200-day interval). Multinomial logistic regression for repeated measures was used to identify predictors of transitioning between different care engagement states including demographics, HIV indicators, substance use and the modified Quan Charlson Comorbidity Index (QCCI).

**Results:** Among 11,064 PWH (median age 47 years, 72% male, 63% NH Black, 48% ≥10 years since HIV diagnosis, 75% VS), the probability of remaining engaged was 85%, the probability of disengagement was 14%. After one 200-day period of disengagement (short-term disengagement), the probability of re-engagement was 55%, 21% after two periods (medium-term disengagement), and 4% after ≥3 periods of disengagement (long-term disengagement). During the observation period 14% of PWH transferred their care; 4% died. Factors associated with short-term disengagement compared to those who remained engaged included younger age, race/ethnicity, male, not on ART at baseline, a QCCI score of 0, private insurance, not VS and CD4 < 500 cells/μL (p-values < 0.05). Medium-term disengagement was associated with younger age, not being on ART, lack of permanent/stable housing, any substance use, public insurance, and CD4 < 500 cells/μL (p-values < 0.05). Predictors of long-term disengagement included increasing age, being NH White, not being on ART, lower QCCI score, lack of permanent/stable housing, and being VS (p-values < 0.05).

**Conclusion:** Among a cohort of PWH who linked to care, we found the probability of re-engaging in care decreased with each additional 6-month interval and factors associated with disengagement varied based on the duration out of care. Identifying PWH at risk of early disengagement may minimize the cyclic nature of care and improve long-term HIV care continuum outcomes.

**RRR (95% CI) for the multinomial regression for the transitions from engaged in care to short-term disengaged vs remaining in care (left panel), from short-term disengaged to medium-term disengaged vs returning to care (center panel), or from medium-term disengaged to remaining in long-term disengaged vs returning to care (right panel).**
Background: In 2016, Blantyre District had the highest adult HIV prevalence in Malawi (17% overall; 22% in women) and the lowest viral suppression rate (60%). In response, the MOH expanded prevention and treatment strategies. We hypothesized that social venues patronized by people with high sexual partnerships rates could identify sub-groups currently missed.

Methods: We conducted cross-sectional bio-behavioral surveys of representative samples of individuals seeking care in government clinics (n=2313) and social venue patrons (n=1802) Jan-Mar 2022. Clinics were randomly selected from government clinics providing HIV testing. Venues were randomly sampled from urban and rural strata with oversampling of rural venues. Sampling weights were based on 2-stage sampling probabilities. Acute infections were identified by pooling dried blood spots from persons with an HIV- rapid test.

Results: Compared to the clinic population, the venue population was more likely to: be male (68% vs 28%); aged >25 years (61% vs 51%); unmarried (62% vs 40%); drink alcohol daily (43% vs 8%); have more sexual partners in the last year (mean 16 vs 2); report a new sex partner in the past 4 weeks (42% vs 14%); and report transactional sex (52% vs 12%). HIV prevalence (Table 1) was higher among the venue population (19% vs 9%); the proportion HIV+ suppressed was similar (78%). Among women recruited at venues, prevalence increased by age: 0% among age 15-17 to 41% among age 18-21. At venues, factors associated with HIV infection include female sex (39% vs 10%); having a new partner in the past 4 weeks (28% vs 13%) and transactional sex (25% vs 13%). Acute and recent infections were uncommon. Clinic participants who reported visiting venues were less likely to have a suppressed viral load than other PLHIV clinic participants (53% vs 81%). Among both populations, reporting a genital sore in the past 4 weeks was associated with non-suppression (40% vs 20% in clinic; 48% vs 20% in venues).

Conclusion: Lower HIV prevalence and greater viral suppression suggests that Blantyre’s HIV epidemic is slowing. Strategies to further reduce transmission should include outreach to venues with higher prevalence of unsuppressed infection and to young women at venues. Testing for acute or recent infection yielded few cases and thus did not provide sufficient value to warrant the cost.

Table 1: Proportion of HIV clinical Outcomes by clinic and venue

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Clinic</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2313</td>
<td>1802</td>
</tr>
<tr>
<td>HIV Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% HIV Negative</td>
<td>91.7</td>
<td>90.8</td>
</tr>
<tr>
<td>% HIV Positive</td>
<td>8.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Among HIV+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Suppressed</td>
<td>78.0</td>
<td>78.1</td>
</tr>
<tr>
<td>% Not Suppressed</td>
<td>22.0</td>
<td>21.9</td>
</tr>
<tr>
<td>Among Not Suppressed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>% Acutely infected</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>% Recently Infected</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>% w/ Chronic</td>
<td>95.4</td>
<td>96.0</td>
</tr>
</tbody>
</table>

Infection suppression = viral load <200 copies/mL

1288 COMMUNITY-ENGAGED PrEP DEMONSTRATION PROJECT FOR MSM IN CHINA

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Background: Financial cost, societal stigma, and suboptimal community engagement delayed the scale-up of Pre-Exposure Prophylaxis (PrEP) among key populations in China. To overcome these barriers, we developed a CBÖ-clinic hybrid model to deliver PrEP.

Methods: The PrEP demonstration project was carried out in two cities (Guangzhou and Wuhan) to provide free PrEP, guided by Community-based participatory research, in which researchers and community stakeholders engage as equal partners. In our model, social workers from community-based organizations (CBO) lead the social media-based recruitment and implementation of the project, such as developing the promotion strategies on the WeChat app or public account, liaising with potential participants, conducting pre-screening, and referring the participants to the clinic for lab testing, while healthcare providers lead the PrEP prescription and monitoring. After enrollment, social workers offered both picking-up or mail delivery of PrEP.
to participants to make the services more convenient. Baseline demographic data and the results of our CBPR strategies were summarized.

**Results:** From September 2021 to September 2022, PrEP and STI self-testing services were delivered to 675 participants, 391 (58%) in Guangzhou and 284 (42%) in Wuhan. Overall, 656 (97.2%) of the participants were cisgender men. 653 (96.8%) of the participants were self-identified as MSM (93.3% MSM and 3.3% MSMW). The median age was 28.8. In the 3 months follow-up survey, among those who reported their regimen, 21% (55/260) of participants reported on-demand regimen. The fully-adherent (self-reported taking 6–7 pills per week on a daily regimen) rates were 97% (200/205), 96% (120/125), and 97.2% (35/36) at months 3, 6, and 9, respectively.

The results of our CBPR strategies suggest that 1286 candidates have registered for interest through CBO’s public account, most of our participants (339, 79.9%) learned PrEP from the community, 613 (90.8%) participants started PrEP after enrollment, and 233 (38%) participants are delivered PrEP by mailing. The detailed strategies and statistics are shown in Figure 1.

**Conclusion:** Findings from our demonstration project suggest that MSM in China are likely to be incentivized by the community. A hybrid CBO and clinic-based model is an effective way to deliver PrEP, as in-person visit provides professional advice, and mailing promotes the accessibility of PrEP. The mobilization of local CBO that closely works with the community can engage the community by developing people-centered services.

**Figure 1. Our community engagement strategies and results**

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**1009 RANDOMIZED TRIAL LEVERAGING ELECTRONIC HEALTH RECORD DATA TO INCREASE HIV PrEP UPTAKE**

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1Kaiser Permanente Northern California, Pleasanton, CA, USA, 2Harvard Medical School, Boston, MA, USA, 3Kaiser Permanente San Francisco Medical Center, San Francisco, CA, USA

**Background:** We previously developed and validated a prediction model that used 44 electronic health record (EHR) data elements to identify patients who are at increased risk of HIV diagnosis and not using preexposure prophylaxis (PrEP). Implementing this EHR-based model in primary care has potential to increase linkage to PrEP care among patients likely to benefit from PrEP.

**Methods:** We conducted a randomized controlled trial of a clinical decision support intervention for PrEP at Kaiser Permanente San Francisco from June-November 2021. Adult primary care providers (PCPs) were randomized to usual care or intervention arms. PCPs who also provide care to people with HIV (PWH) were balanced between trial arms. PCPs in the intervention arm were alerted via EHR-based secure email messages about patients with elevated model-generated risk scores (3-year risk of HIV of ≥0.2%) and upcoming in-person or virtual visits, with prompts to discuss HIV prevention and PrEP. Providers only received alerts for patients aged ≥18 years with no previously documented HIV diagnosis or PrEP prescription. The primary study outcome was the 90-day cumulative incidence of linkage to PrEP care, defined as a PrEP diagnosis code during a clinical encounter (reflecting a PrEP discussion), PrEP referral, or PrEP prescription fill. We report the intervention hazard ratio (HR) and 95% CI from Cox regression models using robust standard errors to account for clustering by provider, with adjustment for and stratification by providers who care for PWH.

**Results:** 121 PCPs were randomized, including 13 with PWH on their panels, with 5051 eligible appointments (2,471 intervention, 2,580 control). The median age of eligible patients was 39 years (IQR 31-51), with 95.4% men and 42.6% non-Hispanic White, 18.7% non-Hispanic Black, 15.1% Hispanic, and 14.6% Asian. There was a nonsignificant increase in PrEP linkage in the intervention arm (6.0% vs 4.5%, HR 1.31, 95% CI 0.84, 2.0). There was a significant interaction by HIV provider status (see Figure 1), with an intervention HR of 2.59 (95% CI 1.30, 5.16) among providers with PWH on their panels and 0.89 (95% CI 0.59, 1.35) for those without (p-interaction < 0.001).

**Conclusion:** A low-intensity intervention that leveraged an EHR-based HIV risk prediction model substantially increased linkage to PrEP care after in-person and video visits among PCPs who also care for PWH. More intensive interventions may be needed to increase PrEP linkage among PCPs less familiar with PrEP and HIV care.

Figure 1. Kaplan-Meier-based cumulative incidence curves for PrEP linkage among intervention and control providers, stratified by PCPs with (left) and without (right) PWH on their panels.

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**1090 COSTS OF PROVIDING PHARMACY-INITIATED PrEP IN KENYA: FINDINGS FROM A PILOT STUDY**

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**Background:** Provision of oral HIV pre-exposure prophylaxis (PrEP) at pharmacies in high HIV prevalence settings can expand access to PrEP and increase convenience to clients; however, implementation costs are uncertain.

We conducted activity-based microcosting to analyze costs associated with this novel PrEP delivery model within an ongoing pilot study evaluating pharmacy-based PrEP delivery in Kenya (CT.gov: NCT04558554).

**Methods:** Trained pharmacy providers at five private pharmacies screened interested clients (≥18 years) for HIV risk and medical safety, conducted HIV testing (using oral self-tests), and dispensed PrEP to those eligible with remote clinician oversight. Costs (2021 USD) were collected from the provider perspective using study budgets, expenditure records, and staff interviews. We interviewed pharmacy owners to obtain financial and economic costs associated with pharmacy overhead, including salaries. We conducted time-and-motion observations of PrEP initiation and refill visits at the pilot pharmacies and examined pharmacy records. We categorized costs into care components of PrEP delivery (HIV risk screening, counseling, HIV testing, prescribing/dispensing) and excluded research costs.

**Results:** From July to October 2021, we conducted 64 time-and-motion observations of clients initiating (n=15) and continuing (n=49) PrEP at pharmacies. Pharmacy PrEP initiation visits took providers a median of 36 minutes (IQR 27-38), with HIV risk screening accounting for a third of this time (median: 12 minutes, IQR 11-17); continuation visits were ~10 minutes shorter (median: 24 minutes, IQR 18-35), with HIV risk screening accounting for a third of this time. The median financial cost for pharmacy providers to deliver PrEP per client was $7.70 per month (IQR $6.72-$9.41) at initiation and $19.86 per 3 months (IQR $17.21-$21.74) at continuation visits, with PrEP drugs accounting for the greatest proportion of costs. The monthly per client cost of pharmacy PrEP initiation visits was lower
than continuation visits ($6.62, IQR $5.47-$7.29), which account for the majority of clients’ visits.

**Conclusion:** Daily oral PrEP can be delivered at reasonable costs at private pharmacies in Kenya, comparable to PrEP delivery costs at public health facilities. Improving efficiencies in pharmacy PrEP delivery (e.g., provider multitasking) may help decrease pharmacy provider time, resulting in potential cost savings. These estimates can inform policy discussions around PrEP scale-up strategies in Kenya and similar settings.

**1091 CLIENT PREFERENCES FOR PR EP REFILLS AT FACILITIES VS PHARMACIES: A PILOT IN KENYA**

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**1Partners in Health Research and Development, Thika, Kenya, 2University of Washington, Seattle, WA, USA, 3Kenya Medical Research Institute, Thika, Kenya, 4Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 5National AIDS and STI Control Program, Nairobi, Kenya, 6Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya**

**Background:** In many high HIV prevalence settings, delivery of pre-exposure prophylaxis (PrEP) in public health facilities is challenged by understaffing, long wait times, and stigma. Expanding PrEP delivery to private pharmacies may help mitigate these barriers. To assess whether current PrEP users would choose to refill their PrEP prescription at a private pharmacy if given the option, we pilot-tested such a model in Kenya.

**Methods:** At two public health facilities in Kiambu County, we recruited adult (>18 years) clients newly initiating PrEP. Once enrolled, clients were given the option to refill PrEP at a public health facility (free) or at one of three nearby private pharmacies (for a fee of 300 Kenyan Shillings, or ~$5.20 US Dollars). Pharmacy providers at pilot pharmacies were trained on PrEP delivery, including how to counsel on HIV risk and PrEP adherence, assess medical safety, complete HIV testing, and refill prescriptions. At enrollment, we asked clients their preferred location for refilling PrEP. We then followed clients for up to seven months and assessed whether and where clients chose to refill their PrEP prescription.

**Results:** From November 2020 to October 2021, we screened 125 clients and enrolled 106. Among enrolled clients, the median age was 31 (IQR 26-38), 59% (n=63) were female, 67% (n=71) were married, and 49% (n=52) were in an HIV serodifferent relationship. At enrollment, clients’ preferred refill location was split between public health facilities (55%, n=58) and private pharmacies (45%, n=48). Over 292 total client-months of observation (median: 1 months per client, IQR 1-4), 42% (n=44) of clients refilled PrEP at least once, and only 3 (3%) clients refilled PrEP at a pilot pharmacy. There was no difference in PrEP continuation (<p=0.05) based on clients’ stated preference for PrEP refill location.

**Conclusion:** Few clients who initiated PrEP at a public health facility in this pilot opted to refill their PrEP prescription at a private pharmacy, despite over half stating a preference for pharmacy-based refills. This data suggests that once PrEP clients in Kenya have overcome barriers to initiate PrEP at a facility, continuation at this location may be easier than at a new location. Additional research is needed to understand drivers of PrEP refill location choice (e.g., cost, trust in service provider) and test implementation strategies (e.g., vouchers) that might enable clients to select their preferred refill location.

**1092 MALE CIRCUMCISION IN BOTSWANA: EFFECT OF QUALITY IMPROVEMENT ON ADVERSE EVENT RATES**

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**Background:** Voluntary medical male circumcision (VMMC) has been shown to reduce heterosexual human immunodeficiency virus (HIV) transmission. In 2009, Botswana, a high HIV burden country in southern Africa, rolled out its VMMC program to expand existing HIV preventive strategies. However, an adverse event rate of 6.7% was recorded for the program in 2017. A quality improvement team was introduced to help reduce the adverse event rate. Data on the impact of the team in reducing adverse event rates are limited.

**Methods:** A quasi-experimental study was conducted using national data extracted from monthly district reporting tools. Interrupted time series analysis was used to compare the trend and magnitude of adverse event rates for the day two, day seven and day forty-two, routine follow-ups in males aged 10 years and older. The comparison was done two years before (April 2015 to March 2017) and two years after (April 2017 to April 2019) the introduction of the quality improvement team. The most common adverse events by age, type, and severity between April 2015 and April 2019 were also reported.

**Results:** After the introduction of the quality improvement intervention, the day two adverse event rates insignificantly decreased by 0.05% (p=0.099, 95% CI=-0.0012, 0.0001), the day seven adverse event rates significantly reduced by 0.08% (p=0.0175, 95% CI=-0.0014, 0.0001) and the day forty-two adverse event rates insignificantly declined by 0.1% (p=0.148, 95% CI=-0.0226, 0.0035). Between April 2015 and April 2019, 1175 adverse events were reported and majority (68.5%) of these adverse events occurred in the 10-14 years age category. Most of these adverse events were mild (73.8%), and infections were the most common type of adverse event (45%).

**Conclusion:** The trend and magnitude of the day two and day forty-two adverse event rates did not significantly change with the introduction of the quality improvement team, but the day seven adverse event rates changed significantly. Therefore, the quality improvement process had a minimal clinically significant effect on the trend and magnitude of adverse events. Infections were the most common type of adverse event thus highlighting the need for increased infection control measures within the VMMC program, especially for the 10-14 years individuals.

**1093 HIV OUTCOMES AFTER EXTENDED 12-MONTH SCRIPTS FOR ART DURING COVID-19 IN SOUTH AFRICA**

Lara Lewis1, Yukeshwara Sookrajh2, Johan van Der Molen1, Thokozani Khubone1, Phlelelani Sobiso1, Riona Govender3, Sifiso Phakathi3, Munthra Maraj3, Peter Mogere4, Mary Mugambi5, Jared M. Baeten2, Kenneth Ngure6, Katrina F. Ortblad4

1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA, 3Kenya Medical Research Institute, Thika, Kenya, 4Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 5Medical Research Council, Pietermaritzburg, South Africa, 6National AIDS & STI Control Programme, Nairobi, Kenya

**Background:** There is an urgent need for more efficient models of differentiated anti-retroviral therapy (ART) delivery, with the World Health Organization and PEPFAR calling for evidence to guide whether 12-monthly ART prescriptions and clinic review (12M scripts) should be recommended in global guidelines. We assessed the association between 12M scripts (allowed temporarily during the COVID-19 pandemic) and clinical outcomes in South Africa.

**Methods:** We performed a retrospective cohort study using routine, de-identified data from 59 public clinics in KwaZulu-Natal. We included PLHIV aged >18 years with a recent suppressed viral load (VL), and who had been referred from their clinic into a community ART delivery programme with a standard 6-month prescription and clinic review (6M script) or a 12M script.

In the community ART programme, PLHIV collected ART every two months at external pick-up points, before returning to the clinic after 6 or 12 months for a new script. We used multivariable modified Poisson regression, accounting for clinic clustering, to compare 12-month retention-in-care (not >90 days late for any visit) and viral suppression (<50 copies/ml) between 6M and 12M script groups.

**Results:** Among 27,148 PLHIV referred for community ART between Jun-Dec 2020, 42.6% received 6M scripts and 57.4% 12M scripts. The median age was 39 years (interquartile range [IQR] 33-46) and 69.4% were women. Age, gender, prior community ART use and time on ART were similar in the two groups (Table). However, a larger proportion of the 12M script group had a dolutegravir-based regimen (60.0% versus 46.3%). The median (IQR) number of clinic visits in the 12 months of follow-up was 1(1-2) in the 12M group and 2(2-3) in the 6M group. Retention at 12 months was 94.6% (95% confidence interval [CI] 94.2%-94.9%) among those receiving 12M scripts and 91.8% (95% CI 91.3%-92.3%) among those with 6M scripts. 16.8% and 16.7% of clients in the 12M and 6M scripts groups were missing follow-up VL data, respectively. Among those with VLs, 90.4% (95% CI 89.9%-91.0%) in the 12M group and 88.9% (95% CI 88.3%-89.5%) in the 6M group were suppressed. After adjusting for age, gender, ART regimen, time on ART and prior community ART use, retention (adjusted risk ratio [aRR]: 1.03, 95% CI 1.01-1.04) and suppression (aRR: 1.02(1.01-1.03) were higher with 12M scripts.

**Conclusion:** COVID-19 led to temporary introduction of 12M scripts in South Africa. Wider use could reduce clinic visits without negative impacts on short-term clinical outcomes.
Table: Baseline characteristics of clients referred for community ART delivery between Jun-Dec 2020, split by baseline ART prescription length

<table>
<thead>
<tr>
<th>Group</th>
<th>ART duration</th>
<th>N</th>
<th>Median age (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 months starts</td>
<td>12 months starts</td>
<td>12 months starts</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23,813 (77.5%)</td>
<td>30,720 (72.4%)</td>
<td>31.9 (18.4-49.7)</td>
</tr>
<tr>
<td>Female</td>
<td>26,187 (22.5%)</td>
<td>11,280 (27.6%)</td>
<td>31.9 (18.4-49.7)</td>
</tr>
</tbody>
</table>

VIRAL SUPPRESSION TRAJECTORIES DESTABILIZED AFTER COVID-19
AMONG US PEOPLE WITH HIV
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Background: Disruptions in clinical services during the COVID-19 pandemic could compromise past progress towards meeting U.S. Ending the HIV Epidemic (EHE) goals. We examined changes in the proportion with virologic suppression (VS) before and since the onset of COVID-19 in a multi-site U.S. cohort of people with HIV (PWH) using an interrupted time series design.

Methods: We assessed VS (< 200 copies/mL) trajectories 1/1/2018-1/1/2022, comparing trends before and after March 1, 2020 at 8 HIV clinics within the U.S. Center for AIDS Research Network of Integrated Clinical Systems ("CNICS"). Hierarchical mixed-effects logistic regression and interrupted time series analyses examined changes in the trend (i.e., slope) of VS over time, and maximum likelihood estimation was used to account for missing VS data among those lost to follow-up (LTFU) post-COVID-19. Analyses were adjusted for demographics, site, CDC transmission group, CD4 nadir, VS, time on ART.

Results: Data from 17,999 participants were included, providing a total of 120,918 VS assessments. Median age was 53 (interquartile range 42-61); 19% were female. VS declined among those lost to follow-up (LTFU) post-COVID-19. Analyses were adjusted for demographics, site, CDC transmission group, CD4 nadir, VS, time on ART.

Conclusion: Previous gains in VS slowed during the COVID-19 pandemic among PWH in a multi-site network of U.S. HIV clinics. Known disparities in VS according to housing status remain unchanged, but VS disparities worsened for PWH who were women, PWID, or Black. Changes in VS trends could be related to socioeconomic impacts of the pandemic, insurance lapses, reduction of in-person clinic services, fear of coming to clinics, or other factors. Renewed investment in HIV public health and clinical services will be vital to achieve the U.S. EHE goals following COVID-19, with additional targeted interventions to support key populations with persistent or worsening disparities needed.

HIV CARE DURING THE SARS-CoV-2 PANDEMIC IN BLACK PEOPLE WITH HIV IN THE UK
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CoV-AFRICA study group
'King’s College Hospital NHS Foundation Trust, London, United Kingdom, 'King’s College London, London, United Kingdom, 'Guy’s and St Thomas’ NHS Foundation Trust, London, United Kingdom, 'University College London, London, United Kingdom, 'St George’s University Hospitals NHS Foundation Trust, London, United Kingdom, 'University College London, London, United Kingdom, 'Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, 'Newcastle Hospitals NHS Foundation Trust, Newcastle, United Kingdom, 'Chelsea and Westminster Hospital, London, United Kingdom, 'AIDS Advocacy Foundation, London, United Kingdom, 'Royal Free Hospital, London, United Kingdom

Background: The COVID-19 pandemic disproportionally affected black communities but the impact on HIV care in this group remains poorly understood. We evaluated measures of HIV care during the COVID-19 pandemic in the GEN-AFRICA cohort of black people with HIV living in the United Kingdom.

Methods: We evaluated interruptions to HIV care during the COVID-19 pandemic (01/2020-09/2022) in the GEN-AFRICA cohort of nine UK clinics who provided HIV outcomes for >80% of their participants. We ascertained death, transfers of care, loss to follow up for >12 months, the highest HIV virus load, and interruptions to antiretroviral therapy (ART). We evaluated factors associated with the composite outcome of HIV viraemia (virus load >200 c/mL) and/or an ART interruption using logistic regression analysis; factors associated (P < 0.1) in univariable analysis were included in the multivariable model. We also summarized reasons for ART interruptions where recorded.

Results: On 01/01/2020, 2321 GEN-AFRICA study participants (mean age 51.3 years; 55.8% women; pre-pandemic current/nadir CD4 of 500/247 cells/mm³ and HIV RNA < 200 c/mL in 92.3%) were under active HIV follow up. Thirty (1.3%) subsequently died, 24 (1.0%) transferred care, and 48 (2.1%) became lost to follow up; 523 (22.7%) had a documented HIV VL >200 c/mL, and 24 (1.0%) subsequently died, 259 (11.2%) had a documented HIV VL >200 c/mL and being vaccinated against SARS-CoV-2 were associated with the composite outcome of HIV viraemia (virus load >200 c/mL) and/or an ART interruption using logistic regression analysis; factors associated (P < 0.1) in univariable analysis were included in the multivariable model. We also summarized reasons for ART interruptions where recorded.

Conclusion: During the COVID-19 pandemic, one in seven black individuals with HIV experienced an ART interruption and/or HIV viraemia. Pre-pandemic measures of suboptimal engagement in care, pandemic restrictions, and wider health beliefs as reflected by SARS-CoV-2 vaccination status, contributed to these undesirable HIV outcomes.
1096 DISRUPTIONS IN HEALTH CARE AMONG MWCCS PARTICIPANTS DURING THE COVID-19 PANDEMIC

Jenni Wise1, Lorie Benning2, Mackey Friedman3, Tracey Wilson4, Catalina Ramirez2, Adaora Adimora2, Bradley Aouizerat5, Anjali Sharma2, Matthew Mimiaga2, Anandi N. Seth2, Michael Planken2,6, Margo Cohen2, Deborah Jones Weiss2,7, Gypsyamber D’Souza8, Mirjam Colette-Kempf9

Combined Cohort Study (MWCCS) and the Women’s Interagency HIV Study

Combined Cohort Study (MWCCS) Research Group

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Background: The COVID-19 pandemic resulted in disruptions to health care services. Vulnerable populations, including people living with HIV (PLHIV), may have experienced unique challenges when accessing medical care. The objective of this study was to evaluate the impact of social disruptions on health care visits among Multicenter AIDS Cohort Study/Women’s Interagency HIV Study Combined Cohort Study (MWCCS) participants.

Methods: A survey collecting data on missed health care visits and social disruptions (i.e., disruptions in employment, childcare, financial support, housing, and health insurance) during the pandemic was administered via telephone to MWCCS participants 1-3 times from March and September 2020.

Logistic regression models adjusted for sociodemographics and HIV-status were used to test the association between social disruptions and three medical care interruption outcomes (i.e., missed healthcare appointment, interruption of mental health care, and interruption of substance use care).

Results: Surveys (n=10,076) were conducted among 2238 PLHIV (61% women) and 1427 people living without HIV (PLWoH) (41% women). Overall, 42% of participants reported disruptions in health care with no significant difference by HIV status. Among participants receiving mental health care services and substance use treatment, 52% and 36% reported interruptions of care, respectively. Participants reporting ≥ 2 social disruptions were more likely to report missed health care appointments (adjusted odds ratio [aOR]: 1.81, 95% confidence interval [CI]: 1.5-2.13), and interruptions in mental health care (aOR: 2.42, 95% CI: 1.8-3.17) or substance use treatment (aOR: 1.97, 95% CI: 1.26-3.09), compared to those reporting no disruptions. Participants who were unemployed were more likely to miss health care appointments (aOR:1.46, 95% CI: 1.25-1.71) and report disruptions in mental health care (aOR: 2.02, 95% CI: 1.54-2.66) compared to those who were employed. PLHIV reporting ≥ 2 social disruptions were at increased risk for missed health care appointments (aOR 1.92, 95%CI: 1.56-2.36) and disruptions in mental health care (aOR: 2.54, 95% CI: 1.83-3.33 (Table 1).

Conclusion: Social disruptions as a result of the COVID-19 pandemic have adversely impacted the receipt of health care among PLHIV and PLWoH, including the receipt of treatment for mental health and substance abuse. Providing childcare, financial support, housing, and health insurance may reduce disruptions in care and improve health outcomes.

1097 UTILITY OF DIGITAL SOLUTIONS IN SUSTAINING ACCESS AND IMPROVING EFFICIENCY OF HIV VL

Ugwuoghere Omo-Emmanuel1, Victor Obianeri1, Omosaewo Oyelaran1, Helina Meri2, Abije Kalaivo3, Dolapo Oyundehi1, Rachel Goldstein1, Jason Williams1, Timothy Yakuba1

1US Agency for International Development Nigeria, Abuja, Nigeria, 2United States Agency for International Development, Washington, DC, USA, 3International Center for AIDS Care and Treatment Programs, Abuja, Nigeria

Background: Coronavirus Disease 2019 (COVID-19) pandemic disrupted routine program implementation worldwide with significant impact on quality and extent of technical oversight of implementation. Diverse digital reporting solutions and online meetings were some strategies designed to bridge program implementation supervision and reporting gaps worldwide. This paper evaluates usefulness and efficiency of digital solutions deployed by USAID Nigeria to ensure adequate oversight to sustain access and reporting of HIV viral load (VL) services.

Methods: To promote accountability and encourage peer-to-peer review and learning among USAID Implementing Partners, daily reporting via digital platforms and virtual weekly peer-review meetings were introduced. This enabled USAID team to monitor IPs’ performance at health facilities and during community VL drives against set targets of 100% and 95% patient VL coverage and suppression (VLC/S) respectively. The platforms include National Laboratory Information Management System, remote sample login and Google-based VL Status and Daily Lab Performance dashboards. This study assesses uptake of VL services and clinical outcomes in 16 states of Nigeria between October 2019 through March 2021 during various levels of COVID-19 lock down.

Chi Square test was used to compare the pre-COVID (October 2019-March 2020), during lockdown (April 2020-September 2020) and post-COVID lockdown (October 2020-March 2021) performances at 95 confidence interval and < 0.05 level of significance.

Results: Significant improvements in VL indicators were reported among eight USAID partners across 16 states. Pre-COVID, 591,906 clients on treatment were eligible for VL monitoring. 455,099 were tested and had documented VL results with a 76.9% and 89% VLC/S. During COVID-lockdown, 685,915 became eligible for VL monitoring, 531,371 had documented VL results, with 77.5% and 90% VLC/S. VLC/S increased to 93% each post-COVID lockdown, when 771,149 had documented VL out of 833,463 eligible. There was a significant increase number of clients on treatment who became eligible for VL test and had documented VL results and suppression from pre- during-COVID, and post-COVID lockdown (p = 0.001).

Conclusion: Digital solutions deployed by USAID were instrumental to sustaining service delivery with significant growth in access and efficiency to HIV VL services in 16 States in Nigeria despite impact of COVID-19. Program managers should continue to explore cost-efficient innovative approaches for program oversight.

HIV Viral Load Performance during Different Period of COVID-19 Response

The adjusted odds ratio of disruptions by number of social disruptions experienced and HIV status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PLHIV (n=1279)</th>
<th>PLWoH (n=1427)</th>
<th>PLHIV (n=1279)</th>
<th>PLWoH (n=1427)</th>
<th>PLHIV (n=1279)</th>
<th>PLWoH (n=1427)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social disruption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
</tr>
<tr>
<td>2</td>
<td>1.92 (1.56-2.36)</td>
<td>1.92 (1.56-2.36)</td>
<td>1.92 (1.56-2.36)</td>
<td>1.92 (1.56-2.36)</td>
<td>1.92 (1.56-2.36)</td>
<td>1.92 (1.56-2.36)</td>
</tr>
</tbody>
</table>
1098 MITIGATING THE IMPACT OF COVID-19 ON HIV VIRAL LOAD ACCESS AMONG KEY POPULATIONS

Ughweroghe Omo-Emmanuel1, Victor Obiarena2, Abije Kalaiwo2, Omosalewa Oyelaran1, Jason Williams3, Timothy Yakubu4, Mark Akhigbe5, Usman Haliru5

1US Agency for International Development Nigeria, Abuja, Nigeria, 2United States Agency for International Development, Washington, DC, USA, 3International Center for AIDS Care and Treatment Programs, Abuja, Nigeria, 4Heartland Alliance LGTie, Uyo, Nigeria, 5Society for Family Health, Yola, Nigeria

Background: Monitoring of HIV-infected individuals on antiretroviral treatment requires periodic viral load (VL) measurements to ascertain adequate response to treatment. While plasma VL is widely available in health facilities, it is difficult to access among key populations (KPs) due to their high mobility and sophisticated sample storage and transport requirements, which are not available for community VL sample collection. Use of Dried Blood Spot (DBS) VL measurement has shown promise as an alternative to plasma specimens for KPs. Studies to investigate the performance of DBSVL quantification against the standard plasma VL assay have proven to be within acceptable range. DBSVL was introduced for sample collection among KPs when it became difficult to safely and appropriately collect, store and transport samples during COVID-19 lockdown. This study assessed the usefulness of the use of DBSVL deployed by USAID to ensure access to HIV VL services among KPs in 7 states of Nigeria during COVID-19 lockdown

Methods: To mitigate the impact of COVID-19 lockdown, virtual trainings were conducted for one-stop-shops and community VL champions of USAID partners providing KPs services in seven states of Nigeria on DBS sample collection, storage and transportation and remote test ordering was activated for service providers. Standard operating procedures and job aids were deployed to points of service and laboratory equipment were verified for DBSVL testing. VL sample collection rate (SCR), VL coverage (VLC), VL suppression (VLS), turnaround time (TAT) and cost savings for the program between March 2019 and February 2021 were compared using the two-sample independent t test pre-COVID (March 2019–February 2020) and during-COVID lockdown (March 2020–February 2021) at 95 confidence interval and < 0.05 level of significance.

Results: There was a significant increase (p < 0.05) in SCR from 73% to 94%, VLC 44% to 85%, and VLS 78% to 95% pre-COVID to during-COVID respectively despite increase in number of clients eligible for VL. However, the median TAT remained unchanged at 29 days. There was a 60% cost savings for the program due to reduction in consumables needed for sample collection and processing and convenience in sampling among KP clients.

Conclusion: Implementation of DBSVL resulted in increases in both VLC and VLS with an improved TAT for KPs client in seven states of Nigeria. KPs Program implementers should consider introduction of DBSVL sampling among KPs for a better VL access and clinical outcome.

HIV Viral Load Sample Collection, Coverage and Suppression pre- and during-COVID-19

<table>
<thead>
<tr>
<th>Time period</th>
<th>VL Coverage</th>
<th>VL Suppression</th>
<th>TAT (days)</th>
</tr>
</thead>
</table>
| Pre-COVID | March 2019-April 2020 | 93% | 11
| During-COVID | March 2020-February 2021 | 97% | 10

p<0.001

1099 IMPACT OF COVID-19 INDUCED PROGRAM ADAPTATIONS ON HIV SURVIVAL IN THREE COUNTRIES

Vamsi Vasireddy1, Neha Shah2, Allalana L. Esber3, Trevor A. Crowell3, Joseph S. Cavanaugh4, Hannah Kubikua2, Ajay Parikh1, Jonah Maswai5, Valentine Sing’Oei4, Emmanuel Bahamana5, Michael Iroezindu6, Julie A. Ake3

1African Cohort Study (AFCOS) group
2Walter Reed Army Institute of Research, Kampala, Uganda, 3Walter Reed Army Institute of Research, Silver Spring, MD, USA, 4Matheron University Walter Reed Project, Kampala, Uganda, 5Walter Reed Army Institute of Research, Nairobi, Kenya, 6Milken Institute School of Public Health, Washington, DC, USA

Background: HIV infection has high global economic and health impacts with transient reductions in HIV clinic attendance and self-reported anti-retroviral therapy (ART) adherence reported in prior studies. Since viral suppression (VS) is an indicator of ART adherence and effective service delivery, we assessed VS in the context of the COVID-19 pandemic in 3 African countries

Methods: Since 2013, the African Cohort Study (AFCOS) has enrolled individuals 18 years or older with and without HIV, in an approximate 5:1 ratio, at 12 clinics across 5 HIV care programs in Tanzania Uganda, Kenya, and Nigeria. For people living with HIV (PLWH), ART history was extracted from medical records and viral load was assessed at each visit. This abstract assesses VS (<1000 c/ml) before and during the COVID-19 pandemic (categorized into 4 surges and a consolidated non-surge period; defined in Table 1) among PLWH. Tanzania was excluded due to inadequate pandemic data. Logistic regression with generalized estimating equations, clustered by participant, was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) comparing VS before and during COVID-19. Models are adjusted for age, sex, and program.

Results: Of the 1741 study participants, 368 are from Uganda, 1156 are from Kenya, and 217 are from Nigeria; 730 are males, 1011 are females, and 147 are under the age of 30. PLWH were less likely to be virally suppressed during the first surge period (OR 0.85, CI 0.46-1.56), but VS significantly increased during the second surge period (OR 1.95, CI 1.23-3.04) compared to the pre-COVID period. The third and fourth surge periods also saw a higher VS (table 1). Females are more likely to be virally suppressed than males (OR 1.38, CI 1.09-2.29) and PLWH ages 40-49 have higher VS (OR 2.43, CI 1.32-4.48) compared to PLWH under. PLWH at the AFCOS sites in Kenya and Nigeria show lower VS than the Ugandan cohort (ORs 0.46, CI 0.26-0.79 and OR 0.32, CI 0.17-0.60 respectively).

Conclusion: The initial drop in VS may be attributable to reduced clinic access due to lockdowns. Many HIV programs supported by the President’s Emergency Plan for AIDS Relief (PEPFAR) adapted their strategies to serve PLWH by scaling up community ART dispensing and multi-month dispensing (MMD) of ART for stable clients, which could have led to increased VS during the other surge periods. These findings demonstrate sustained progress made by PEPFAR-supported programs against the HIV epidemic amidst the COVID-19 pandemic Table 1: Adjusted Odds Ratios of Viral Load Suppression during COVID Pandemic, AFCOS Sites, March 2020 to June 2022

<table>
<thead>
<tr>
<th>Time period</th>
<th>Reference</th>
<th>Male</th>
<th>Female</th>
<th>Odds Ratio</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-COVID</td>
<td>Reference</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99-1.00</td>
</tr>
<tr>
<td>Surge 1</td>
<td>0.51</td>
<td>0.67</td>
<td>0.80</td>
<td>0.52-1.24</td>
<td></td>
</tr>
<tr>
<td>Surge 2</td>
<td>0.85</td>
<td>1.56</td>
<td>0.88</td>
<td>0.46-1.56</td>
<td></td>
</tr>
<tr>
<td>Surge 3</td>
<td>1.95</td>
<td>3.04</td>
<td>1.95</td>
<td>1.23-3.04</td>
<td></td>
</tr>
<tr>
<td>Non-surge (remaining periods combined)</td>
<td>0.46</td>
<td>0.79</td>
<td>0.32</td>
<td>0.17-0.60</td>
<td></td>
</tr>
</tbody>
</table>

1010 CHANGES IN SERVICE ACCESS AMONG PEOPLE WHO INJECT DRUGS IN PHILADELPHIA, 2018-2022

Tanner B. Nassau1, Kathleen A. Brady1

1Philadelphia Department of Public Health, Philadelphia, PA, USA

Background: The COVID-19 pandemic disrupted HIV prevention and treatment services, especially for structurally vulnerable individuals like many people who inject drugs (PWID). We sought to compare present levels of access to these services to their levels before the pandemic.

Methods: We used data from 2018 and 2022 collected through the National HIV Behavioral Surveillance (NHBS) survey among PWID in Philadelphia. Using generalized linear regression models, we estimated the associations between our exposure (year) and self-reported HIV testing, medical care, SSP access, PrEP use, and drug treatment in the year prior to interview. We calculated adjusted prevalence ratios (aPR) using multivariable models adjusted for age, race/ethnicity, housing stability, and primary injecting drug.
Results: There were 620 participants in 2018 and 604 in 2022 included in analyses. Compared to the 2018 sample, the 2022 sample was significantly older, non-Hispanic Black, and primarily injected drugs other than heroin. A significantly smaller proportion of participants in 2022 had a recent HIV test (57% vs. 71%), visited a health care provider (77% vs. 82%), received sterile needles from an SSP (69% vs. 75%), or participated in a drug treatment program (47% vs. 54%). Between 2018 and 2022, PrEP awareness increased significantly (39% vs. 54%) but PrEP use did not (3% vs. 3%). In adjusted models, an 18% decrease in recent HIV testing was observed between 2018 and 2022 (aPR: 0.82; 95% CI: 0.70-0.96). Among those who reported a recent HIV test, there was an 18% increase in testing in clinical settings observed between 2018 and 2022 (aPR: 1.18; 95% CI: 1.10-1.26). Recent medical care, SSP access, PrEP use, and drug treatment were not associated with year in adjusted models.

Conclusion: Access to a full range of social services is necessary for Ending the HIV Epidemic. These findings indicate that HIV prevention services, particularly HIV testing, among PWID have not rebound fully from the pandemic. Considering this and ongoing outbreaks of HIV among PWID, public health practitioners should closely monitor HIV testing frequency among PWID and prioritize expanding access to low-barrier HIV prevention and care services, especially in non-clinical settings.

1101 COST EFFECTIVENESS OF NIRMATRELVIR/RITONAVIR FOR MILD-MODERATE COVID-19 IN SPAIN
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Tricky Bugs
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Background: A need exists for safe, affordable, and effective antiviral treatments for less severe COVID-19 outpatients that can prevent infection progression, hospitalization, and deaths; shorten the time to clinical recovery; and reduce transmission. In our best knowledge, there are not, so far, cost-effectiveness analysis on oral antiviral COVID-19 drugs in Spain. In our study we aim to evaluate cost-effectiveness of oral nirmatrelvir plus ritonavir in COVID-19 mild to moderate outpatients with at least one risk factor for disease progression in Spain.

Methods: A simulation model was constructed in R, to assess the clinical consequences and costs associated with COVID-19 in a hypothetical cohort of non-hospitalized patients older than 65 years with mild-to-moderate COVID and at least one risk factor for progression in Spain. The intervention assessed was nirmatrelvir plus ritonavir 300 mg plus 100 mg every 12 hours up to 5 days. The comparator was symptomatic treatment with no antiviral drugs against SARS-CoV-2. The study was contextualized in the Spanish National Health System and the perspective of the service provider was adopted. Quality of life adjusted life years (QALYs) was used as a measure of effectiveness. Drug effectiveness was obtained from a literature review. As a cost measure, the retail price of the drugs was used. As a threshold willing to pay, the Spanish Gross National Product per capita was used. A discount of 3% per year was applied on future health effects. We used a decision tree model. A univariate sensitivity analysis and probabilistic sensitivity analysis was performed.

Results: We found that nirmatrelvir/ritonavir yielded an extra 620.89 QALYs compared to a baseline scenario without it, at an increase in cost of 8,630,442 € with an Incremental cost-effectiveness ratio of 144,356.4 €/QALY gained. One way sensitivity analysis and probabilistic sensitivity analysis using Monte Carlo simulations were undertaken and showed that the probability of not being cost-effective was 1 at the current price and willingness to pay threshold. To meet our willingness to pay threshold, nirmatrelvir plus ritonavir 5 days-treatment price should be lowered down to 70 €.

Conclusion: According to our analysis nirmatrelvir/ritonavir is not cost-effective in Spain. The procedure was considered cost-effective in the Spanish National Health System for outpatients older than 65 years with at least one risk factor for COVID progression. A drug price of 70€ per treatment would meet our willingness to pay threshold.

Cost-Effectiveness plane of the Probabilistic Sensitivity Analysis
Table 1. Demographics, clinical characteristics and outcomes by HIV status for patients treated with tecovirimat in NYC via telehealth, June 9 – August 18, 2022

<table>
<thead>
<tr>
<th>Demographics, clinical characteristics and outcomes by HIV status</th>
<th>Total</th>
<th>HIV status (64/75)</th>
<th>HIV status (6/15)</th>
<th>Total (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian, Asian/Black</td>
<td>4 (12.5)</td>
<td>1 (20)</td>
<td>7 (27.5)</td>
<td>20 (25)</td>
</tr>
<tr>
<td>White</td>
<td>12 (37.5)</td>
<td>7 (40)</td>
<td>25 (31.2)</td>
<td>40 (50)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (12.5)</td>
<td>1 (20)</td>
<td>7 (27.5)</td>
<td>20 (25)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CD4 count, median (IQR)</td>
<td>581 (479–736)*</td>
<td>27 (17–32)</td>
<td>581 (479–736)*</td>
<td>27 (17–32)</td>
</tr>
<tr>
<td>Recovery at 30 days post exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>14 (42.5)</td>
<td>5 (100)</td>
<td>19 (76.9)</td>
<td>100 (125)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>20 (62.5)</td>
<td>1 (20)</td>
<td>22 (89.5)</td>
<td>22 (27.5)</td>
</tr>
</tbody>
</table>

*2 patients did not have HCV serostatus documented

N = 64/75 patients treated with tecovirimat in NYC via telehealth, June 9 – August 18, 2022. 1 patient was lost to follow-up, 2 patients were not in New York City, and 3 patients were not diagnosed with monkeypox. 3 patients were not diagnosed with monkeypox. 3 patients were not diagnosed with monkeypox. 3 patients were not diagnosed with monkeypox. 3 patients were not diagnosed with monkeypox.