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This issue of *Topics in Antiviral Medicine* is a special issue that includes the abstracts from the 2021 virtual Conference on Retroviruses and Opportunistic Infections (CROI). This issue is funded and supported by IAS–USA. Below is a sample of how to cite a CROI abstract:


Information on citing presentations from vCROI 2021 is available on the Resources page at CROIconference.org.

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### vCROI 2021 Resources

Resources from CROI 2021, as well as information about CROI 2022 (to be held in Denver, Colorado, USA from February 13 to 16, 2022), can be found at www.CROIconference.org. The vCROI 2021 Resources page includes the following resources and more.

The CROI Program and Information Guide includes information about sessions, speakers, and other details about CROI 2021. In addition to the Special Issue of *Topics in Antiviral Medicine*™ abstracts from CROI 2021 can be viewed in the Abstract eBook and searchable database. Beginning April, 2021, plenaries, interactive sessions, oral abstract presentations, and Science Spotlights™ will be available as webcasts. Archived webcasts from CROI 2014 to 2020 are also available. Visit the resources page of www.CROIconference.org for more details.

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ABSTRACTS

1 PROGRAM COMMITTEE WORKSHOP FOR NEW INVESTIGATORS AND TRAINEES: SESSION OVERVIEW
Serena S. Spudich, Yale 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 presentations will cover both HIV and SARS-CoV-2. The program will begin with a presentation by Dr Frank Kirchhoff on novel aspects of the HIV-1 and SARS-CoV-2 replication cycles, with an emphasis on the similarities and differences between the two viruses. Following this, Dr Galil Alter will cover the immune responses (with a particular focus on B- and T-cell responses) against HIV and SARS-CoV-2. Dr Jürgen Rockstroh will outline the most efficient prevention measures for controlling the COVID-19 pandemic and will review new testing technologies as well as therapeutic strategies and currently available SARS-CoV-2 vaccines. In the next presentation, Jean-Michel Molina will address advances in different biomedical strategies for prevention of HIV transmission, with an emphasis on the recent development in preexposure prophylaxis (PrEP) but also some of the emerging strategies to limit SARS-CoV-2 transmission. Finally, Dr Katharine Bar will review advances in characterizing the size and composition of the replication- and rebound-competent HIV-1 reservoirs as well as highlight several preclinical and clinical approaches for functional or sterilizing HIV-1 cure. By the completion of the workshop, attendees will have achieved a head start toward maximizing the knowledge gained and research ideas arising from vCROI 2021.

2 VACCINE NATIONALISM IS KILLING US: HOW INEQUITIES IN RESEARCH AND ACCESS TO SARS-CoV-2 VACCINES WILL PERPETUATE THE PANDEMIC
Gregg S. Gonsalves, Yale University, New Haven, CT, USA
This presentation will include a conversation among the panelists as they discuss the worldwide struggle for access to antiretroviral therapy and the current battle to ensure everyone is vaccinated against SARS-CoV-2 across the planet. Panelists will discuss how coalitions among activists, affected communities, and scientists were crucial for the fight against AIDS and how similar collaborations are vital now in the face of the COVID-19 pandemic. Panelists will discuss the similarities in the challenges for global access to antiretroviral therapy and SARS-CoV-2 vaccines but also how the speed and breadth of the spread of the novel coronavirus present new obstacles to existing institutions (eg, NIH, WHO, GAVI, WTO) and require solutions at far larger scale and established with greater urgency than accomplished for HIV, tuberculosis and malaria to date.

3 NEUTRALIZING ANTIBODIES AGAINST CORONAVIRUSSES
Pamela J. Bjorkman, California Institute of Technology, Pasadena, CA, USA
Neutralizing monoclonal antibodies against SARS-CoV-2 are being used as therapeutics against COVID-19. The Bjorkman laboratory is investigating the mechanisms of virus neutralization by human antibodies that bind the SARS-CoV-2 spike protein. Using structural techniques, including single-particle cryo-electron microscopy and X-ray crystallography, the laboratory has solved spike trimer-antibody structures that allow classifying antibodies with respect to spike recognition and neutralization. They are also designing and testing mosaic nanoparticles that induce broadly cross-reactive antibodies with the goal of creating a pan-coronavirus vaccine that could protect against SARS-CoV-2 and future coronaviruses with the potential to spill over into humans.

4 LESSONS FROM THE CONCURRENT HIV/AIDS AND COVID-19 PANDEMICS: A TWO-WAY STREET
Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
The concurrent COVID-19 and HIV/AIDS pandemics pose unprecedented societal, economic, and public health challenges to nations around the world. The juxtaposition of the HIV/AIDS and COVID-19 pandemics underscores the priority and urgency to accelerate the development and clinical evaluation of prevention and treatment countermeasures. Lessons learned from the HIV/AIDS pandemic inform the discovery and testing of innovative strategies to prevent, treat, and care for individuals with SARS-CoV-2 infection and COVID-19. Similarly, the COVID-19 pandemic highlights that an effective response to the HIV/AIDS pandemic requires a novel coordinated and collaborative global effort of scientists, industry, and community partners to accelerate basic and clinical research as well as implementation science to operationalize evidence-based interventions expeditiously in real-world settings.

5 STRUCTURES OF SARS-CoV-2 ANTIBODIES INDUCED BY INFECTION AND mRNA VACCINES
Christopher O. Barnes, California Institute of Technology, Pasadena, CA, USA
In the space of less than a year, structural biology uncovered structure-function details for many of the proteins encoded by SARS-CoV-2, the coronavirus that has caused worldwide suffering and more than 1 million deaths since 2019. Remarkably, structures of the SARS-CoV-2 spike trimer were published in March 2020, only about 2 months after the viral sequence was available, aided by previous studies that established how to stabilize coronavirus spikes and the rapid turn-around time for solving structures by single-particle cryo-electron microscopy. Since then, other structures have revealed how spike binds to its angiotensin-converting enzyme 2 (ACE2) receptor, the specificities of polyclonal antibody responses in COVID-19–convalescent individuals, and how monoclonal neutralizing antibodies or designed protein inhibitors bind spike to prevent infection. Taken together, these structures have informed the development of potential therapeutics, including how pairs of monoclonal antibodies are chosen for treatment cocktails and guided structure-based engineering approaches to improve antibody potencies that are effective at lower doses and/or are resistant to viral mutations. With the rapid improvements in microscopes, cameras, and processing techniques, details of individual viral proteins (eg, spikes and ribonucleoproteins) can be resolved in their native context, providing more knowledge for researchers to use against this virus. Here, we will detail studies of neutralizing antibodies against the receptor binding domain induced by both infection by SARS-CoV-2 and by mRNA vaccines. Our data suggest that functionally similar antibodies are raised during vaccination and natural infection, and that the RBDS of spike trimers translated from the mRNA delivered by vaccination adopt both “up” and “down” conformations as observed on structures of trimer ectodomains and trimers on the surface of SARS-CoV-2 virions. Taken together, our work and the work of others illustrate the value of structural biology as a tool to gather information that will aid us in our battle to control the current pandemic and future outbreaks of deadly viruses.
6 EMERGING CONCEPTS IN HIV-1 RESTRICTION
Edward Campbell, Aalborg University Hospital, Aalborg, Denmark

The ability of HIV-1 to replicate in a target cell depends on its ability to evade the activity of cellular proteins, known as restriction factors, which are capable of inhibiting numerous steps of the HIV-1 replication cycle. In this regard, viruses and their hosts are locked in an evolutionary arms race in which selective pressure on both viruses and the host drives the refinement and expansion of genes that facilitate the evasion or maintenance of the antiviral activity of these proteins, respectively. In this talk, I will provide a survey of the restriction factors known to impact HIV-1 replication and the viral mechanisms used to evade these restriction factors. I will specifically focus on the methodologies used to identify and interrogate these interactions, I will also highlight opportunities afforded by our understanding of the restriction factors that can inhibit HIV-1 infection, or the technologies utilized to identify them, that may be used to interrogate virus/host interactions with other viruses, such as SARS-CoV-2, and highlight early efforts in this area.

7 SARS-CoV-2 SPECIFIC AND CROSS-REACTIVE T CELL RESPONSES
Daniela Weiskopf, University of California San Diego, San Diego, CA, USA

Understanding adaptive immune responses to SARS-CoV-2 is important for vaccine development efforts, interpreting disease pathogenesis, and calibration of future pandemic control measures. We have developed HLA class I and class II predicted peptides, collected into "Megapools" (MP), to identify SARS-CoV-2–specific CD4+ and CD8+ T cells in coronavirus disease 2019 (COVID-19) convalescent patients. Importantly, we detected SARS-CoV-2 reactive CD4+ T cells in 40–60% unexposed individuals sampled before 2019, implicating pre-existing cross-reactive T-cell memory. Using human blood samples derived pre-2019, we demonstrated a range of preexisting memory CD4+ T cells that are cross-reactive with comparable affinity to SARS-CoV-2 and HCoV-OC43, HCoV-229E, HCoV-NL63, or HCoV-HKU1. Thus, vaniegated T-cell memory to common cold coronaviruses may underlie at least some of the extensive heterogeneity observed in COVID-19 clinical and subclinical disease.

8 ARTIFICIAL INTELLIGENCE–INSPIRED ANTIBODY ENGINEERING
Sai Reddy, ETH Zurich, Zurich, Switzerland

Machine learning and deep learning are part of a family of tools related to artificial intelligence and represent an emerging field of information and computer science that uses large data sets to extract features and representations. Antibody discovery and engineering is reliant on experimental platforms of high-throughput expression and screening of libraries. Here, I will describe how researchers are using machine and deep learning to assist in antibody engineering experiments and thus move beyond experimental screening. One area that I will highlight in particular is related to deep sequencing of natural antibody repertoires (derived from 8 cells of humans and mice), which has become a promising and powerful tool in basic immunology, immunodiagnostics, and the drug discovery process. However, identification of relevant information in these large datasets remains challenging. I will explain how deep learning is being used to identify patterns of antigen–specificity from antibody repertoires. Approaches such as unsupervised clustering and deep generative modeling are then being used to elucidate the antibody sequence space by generating thousands of novel and functional variants in silico. In addition deep learning is also being used to interrogate and predict antigen–specificity from a massive diversity of antibody sequence space from synthetic antibody repertoires (derived from surface display libraries). With its scalability and capacity to interrogate a vast protein sequence space, deep learning offers great potential for antibody discovery, engineering, and optimization.

9 MAKING SENSE OF STUDY DESIGN DIFFERENCES BETWEEN COVID-19 PLATFORM TRIALS
Lori Dodd, National Institutes of Health, Bethesda, MD, USA

Platform trials are multi-arm studies that allow experimental agents to enter and exit a study over time. Sharing a control arm can provide efficiency gains in terms of a smaller total sample size. Many trialists have recommended platform trials for COVID-19 treatment studies largely due to their potential to reach conclusions faster. However, not all platform trials are created equally. Important design choices that alter study rigor include study endpoints, whether to use a placebo, whether to use response-adaptive randomization, whether to allow comparisons with nonconcurrent controls, how many agents to study, and when to drop/add study agents. In this talk, I will provide a framework to critically evaluate study design decisions behind COVID-19 treatment trials. This framework will be applied to the major trials as a way to rank quality of study results.

10 DESIGN OF CURRENT AND FUTURE COVID-19 VACCINE EFFICACY TRIALS
Holly Janes, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Rapid development and deployment of safe and effective COVID-19 vaccines for the global population is a public health imperative. We will overview the key statistical design elements of the first-generation US-government-funded vaccine efficacy trials, including the rationale and choice of endpoints, success criteria, triggers for analysis, and design adaptations following early evidence of efficacy. The implications of the emerging results for second-generation trials to evaluate additional vaccine candidates and to complete the profile of existing vaccines will be discussed.

11 RAPID GENOMIC SEQUENCING FOR MANAGEMENT OF COVID-19
Kwok-Yung Yuen, University of Hong Kong, Pok Fu Lam, Hong Kong

Rapid target enrichment sequencing documented the first case of reinfection by SARS-CoV-2. The reinfected virus has 24 nucleotides (12 amino acids) difference with one stop codon leading to a deletion of 58 amino acid at orf8. Moreover the reinfected virus is located on a different branch from the first infecting strain on the phylogenetic tree. Retrieval and testing of his initial serum showed that the neutralizing antibody titre of 40 has dropped within 5 months to below 10 at the time of re-infection. Within 3 days after re-infection, his serum antibody level started to rise, and it reached 3200 within 8 days. Besides differentiating re-infection from persistent infection, rapid genome sequencing has been used to demonstrate person-to-person transmission in a family cluster of COVID-19. This technology is also useful for the investigation of hospital outbreak, which led to the refinement of admission SARS-CoV-2 screening strategy. In terms of public health policy, phylogenomics has demonstrated the importance of border control to prevent virus entry and the necessity of stringent social distancing measures to prevent virus dissemination in the community. The close monitoring for virus mutants has led to the discovery of highly transmissible mutants such as the Spike D614G and N501Y and other Spike mutants that may have varying degrees of resistance to remdesivir, therapeutic antibodies, and vaccines.

12 COVID-19: A HOSPITALISED UNWELL PATIENT WITH PNEUMONITIS
Sanjay Bhagani, Royal Free Hospital, London, UK

This case study will focus on clinical presentation and evidence-based management of patients with severe COVID-19. Risk factors for mortality and predictors of severity will be discussed. In addition to best standard of care, we will explore evolving data on the use of antiviral and immune-modulatory therapy.

13 CRITICAL CARE OF COVID-19
Janhavi Athale, Mayo Clinic Arizona, Phoenix, AZ, USA

The novel coronavirus of 2019 (SARS-CoV-2) has resulted in an increased incidence of patients admitted to intensive care units (ICUs). On average 5 percent (ranges of 3-80% have been reported) of patients with COVID-19 require ICU admission. Unfortunately, a vast majority of these ICU patients contribute to the mortality seen with this disease. The chief reason for ICU admission in COVID-19 is hypoxia. Infection with SARS-CoV-2 results in inflammation and damage to the lung parenchyma via the receptor angiotensin-converting enzyme 2 (ACE2) on type II pneumocytes. The resulting lung damage presents as a clinical syndrome: Acute Respiratory Distress Syndrome (ARDS). Thus, the management of COVID-19—associated ARDS has been adopted from prior studies that have assessed interventions in ARDS. Treatment of ARDS remains complex with very few studies demonstrating mortality benefits. In addition to hypoxia associated with COVID-19, the presentation will address 2 additional features of COVID-19: cytokine storm and immunothrombosis or hypercoagulability associated with COVID-19. The cytokine storm, or hyperinflammatory syndrome, associated with COVID-19 has been attributed to the exaggerated immune response to the virus that results in multigorgan dysfunction. Treatment of this hyperinflammatory syndrome has been extrapolated from cytokine release syndrome (CRS) seen in other diseases. The increased incidence of venous thromboembolism (VTE) and additional clotting seen with COVID-19 has resulted in aggressive anti-coagulation strategies in treatment of this disease. We are continuing to obtain new data on COVID-19,
and the management decisions can be very institution- or provider-dependent. The goal of this presentation is to review the current data and outline some of the salient features in treatment of critically ill patients with COVID-19.

**IMAGING VIRAL LIFE CYCLES**

Hans-Georg Kräusslich, Heidelberg University, Heidelberg, Germany

Understanding viral replication and spread for a long time depended on bulk analysis of infected cells and mostly used transformed cell lines in tissue culture. Advances in imaging methods and labeling tools more recently allowed studying individual infection events and specific stages of the infection cycle. This includes analysis of trafficking and morphological changes of individual viral components at high spatial and/or temporal resolution also using primary target cells. In parallel, advances in (cryo) electron microscopy and tomography not only allowed visualizing structural components of the extracellular virion at near atomic resolution but also yielded structural information on viral components inside infected cells and on their interaction with host cell components. On the other hand, development of organoid systems and other 3D culture systems in combination with light sheet microscopy and other methods allowing analysis of virus infection in complex 3D cultures yielded important new insights on viral infections more similar to the real in vivo situation; certain aspects of viral replication and spread could even be studied in living animals, applying 2-photon-microscopy. This overview will summarize and discuss recent advances achieved by improved imaging technologies with a major focus on HIV-1, but also addressing aspects important for other viruses.

**DISPARITIES IN HEALTH: FROM HIV TO COVID-19 AND BEYOND**

James E. Hildreth, Meharry Medical College, Nashville, TN, USA

The 2 ongoing pandemics in the US, COVID-19 and HIV/AIDS, have several parallels including significant racial disparities in disease burden. These parallels will be discussed along with a consideration of the social determinants of health underlying these inequities.

**SARS-CoV-2 EVOLUTION IN POPULATIONS AND INDIVIDUALS**

This highly interactive session begins with a brief overview of the issue or controversy. Each of the scientific experts offer their opinions or observations in a 5-minute summary, followed by a 30-minute spirited discussion among the panel members. The moderator will bring in comments and questions from the audience. This session will address the evolution of SARS-CoV-2 vs HIV at the population level, viral and antibody evolution in populations, the origin and spread of highly transmissible variants of SARS-CoV-2, and the selection of neutralization-resistant SARS-CoV-2 mutants.

**CASE-BASED DISCUSSION ON WEIGHT GAIN IN HIV AND ANTIRETROVIRAL THERAPY**

This interactive session will discuss current controversies about the causes and consequences of weight gain during antiretroviral therapy (ART) using a case-based format. Specific topics that will be covered include: 1) the relative contributions of particular ART drugs/classes and reversal of viral replication/inflammation to weight gain, 2) the pathophysiology of weight gain during ART, 3) the impact of ART-associated weight gain on type 2 diabetes risk, and 4) how these issues are perceived and managed in diverse populations, including children, pregnant women, and people with HIV in low-income countries.

**CONTACT TRACING IN CONTROLLING EPIDEMICS: IS THE JUICE WORTH THE SQUEEZE?**

This interactive session will begin with 3 presentations by experts describing the role, methodology, and ethical issues of contact tracing. Following the presentations, there will be a moderated discussion and responses to comments and questions from the audience.

**LIVE-CELL IMAGING OF HIV-1 NUCLEAR IMPORT, UNCOATING, AND PROVIRUSES**

Vinay K. Pathak, National Cancer Institute, Frederick, MD, USA

The HIV-1 mature conical core, composed of ~250 capsid protein (CA) hexamers and ~12 pentamers, must disassemble (uncoat) and the viral DNA must enter the nucleus before it can integrate into the host genome. For the past 4 decades, retroviral uncoating has been widely believed to occur in the cytoplasm, and some recent studies have proposed that uncoating occurs at the nuclear envelope (NE) just prior to nuclear import. Studies of uncoating have been hampered by an inability to accurately quantify the amount of CA associated with viral reverse transcription/preintegration complexes, and an inability to study rare infectious viral cores in a vast majority of non-infectious viral cores. We recently developed methods to directly label CA with green fluorescent protein (GFP) and track viral cores in infected cells by live-cell microscopy. In addition, we developed methods to identify infectious viral cores that led to the formation of transcriptionally active proviruses. In striking contrast to the prevailing models of nuclear import and uncoating, our results showed that infectious viral cores in the nuclei are intact and complete reverse transcription in the nucleus before uncoating. Recent studies from other groups supporting this model have shown that viral cores in the nucleus are largely intact, that reverse transcription requires an intact capsid and that reverse transcription is completed in the nucleus. We also probed the mechanism of viral core nuclear import and showed that intact viral cores gain nuclear entry through a mechanism involving interactions at the NE with the host protein cleavage and polyadenylation specificity factor 6 (CPSF6). Using GFP as a capsid content marker, we have recently observed that nuclear capsids retain their integrity and maintain a separation between the viral core contents and the nuclear environment until <1.5 hours before integration. These observations fundamentally change our current understanding of HIV-1 post-entry replication events including mechanisms of nuclear import and uncoating as well as reverse transcription, integration, and evasion of innate immunity.

**VISUALIZATION OF HIV-1 CAPSID-DEPENDENT REPLICATION IN VITRO**

Barbie Ganser-Pornillos, University of Virginia, Charlottesville, VA, USA

HIV-1 initiates receptor-mediated entry of the virus core particle into the cytoplasm of a host cell. This core particle is organized by a full-length capsid shell, which houses the viral RNA genome and its associated replicative enzymes. Upon entry, the capsid facilitates reverse transcription, shields the viral nucleic acids from host defense sensors, and coordinates interactions with many different host factors to deliver the genome to the nucleus. A variety of studies now appear to be converging upon a model in which reverse transcription initiates soon after entry but does not become complete until the core is at the nucleus, whereupon the capsid uncoats and releases the pre-integration complex in close proximity to the host chromosomes. In situ mechanistic and structural studies of these processes remain challenging because these operations are executed by individual viral particles deep within cells. Here, I will discuss studies in which we reconstitute endogenous reverse transcription in vitro from HIV-1 cores that are released from permeabilized virions. This cell-free system allowed us to monitor the core structure throughout the entire process of replication and uncovered an important role for the capsid in templating replication. Capsid persistence during reverse transcription appears to be dictated not only by the intrinsic stability of the CA lattice but is also positively and negatively modulated by host factors. We are...
now expanding this reconstitution system to include the nuclear import steps and eventual integration into authentic human chromosomes.

22 LENACAPAVIR (GS-6207): FIRST CLINICALLY ACTIVE LONG-ACTING INHIBITOR OF HIV CAPSID
Tomas Cihlar, Gilead Sciences, Inc, Foster City, CA, USA
A program building on prior extensive structural and functional characterization of HIV capsid and spanning a decade of drug discovery work yielded lenacapavir (LEN; GS-6207), a small molecule inhibitor targeting several critical functions of HIV capsid. LEN binds at a conserved interface between capsid monomers and interferes with protein interactions essential for multiple phases of the HIV replication cycle, including both the assembly and disassembly of capsid core as well as capsid nuclear trafficking. LEN exhibits in vitro antiviral activity at picomolar concentrations against all subtypes of HIV, including strains resistant to other antiretroviral classes. A potent antiretroviral activity of a single dose LEN has been demonstrated in phase 1b viral dynamics study conducted in treatment-naïve people living with HIV, and several ongoing clinical studies are evaluating the efficacy of LEN administered once every 6 months subcutaneously in combination with other antiretroviral agents. In addition, emerging efficacy data from non-human primates support further investigation of LEN as a long-acting agent for pre-exposure prophylaxis.

23 FROM GENOME TO FUNCTION: PHENOTYPIC CHARACTERISATION OF PANDEMIC SARS-CoV-2 VARIANTS
Volker Thiel, University of Bern, Bern, Switzerland
SARS-related coronaviruses have been known for several years to circulate in diverse bat species and they are known to have the potential to infect humans. The zoonotic emergence of SARS-CoV-2 exemplified that these viruses also have pandemic potential. Here I will show how we can reconstruct SARS-CoV-2 in order to obtain molecular clones for functional and phenotypic studies. Based on this novel reverse genetic system it is now possible to rapidly reconstruct SARS-CoV-2 and to phenotypically characterize SARS-CoV-2 variants that emerge during the pandemic in real-time.

24 IMMUNE EVASION STRATEGIES BY SARS-CoV-2: Nsp1 AND BEYOND
Konstantin Sparrer, Ulm University Medical Center, Ulm, Germany
The human innate immune system represents a powerful first line of antiviral defenses. Incoming viral pathogens are quickly detected by dedicated germline encoded sensors, called pattern recognition receptors. For example, intracellular RNA viruses, like the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are detected by RIG-like receptors such as RIG-I and MDA5. Activation of these sensors initiates signaling cascades that ultimately lead to the secretion of different types of interferons (IFNs) and other pro-inflammatory cytokines. Upon binding to their respective receptors, these cytokines induce a transcriptional program setting infected and neighbouring cells in an antiviral state. In parallel, other antiviral mechanisms, such as autophagy, are mounted. Autophagy is capable of targeting viruses and viral components for lysosomal degradation, facilitating their removal from a cell as well as immune recognition. Eventually, activation of the innate immune system stimulates the adaptive immune system. However, successful viral pathogens like SARS-CoV-2 have evolved intricate strategies to evade or even subvert innate immunity.

Recent research has shown that most of the approximately 30 proteins that are encoded by SARS-CoV-2 manipulate and disarm innate immune defenses. Major innate immune antagonists include the non-structural proteins (Nsp) 1 and 3 as well as the accessory proteins ORF3a and ORF6. Nsp1 blocks cellular ribosomes, consequently preventing translation of antiviral proteins including IFNs. Nsp3 attenuates the type-I IFN response by removing the post-transcriptional modifier 5G1S from the immune sensor MDA5 and the transcription factor IRF3. ORF3a was reported to suppress autophagic degradation by blocking the turnover, while ORF6 interferes with nuclear translocation of transcription factors required for the IFN response. Here, I will give an overview on innate immune evasion strategies employed by SARS-CoV-2 and highlight selected molecular mechanisms.

25 SHARED VULNERABILITIES AND DIFFERENT COUNTERMEASURES OF HIV AND SARS-CoV-2
Eric O. Freed, National Cancer Institute, Frederick, MD, USA
Viruses rely heavily on host cellular machinery to replicate. In turn, cells have evolved elaborate defense mechanisms to impede virus replication. Numerous cellular proteins, often referred to as inhibitory or restriction factors, are central components of the innate immune response that serves as the first line of defense against invading pathogens. In recent years, several families of host proteins have been shown to inhibit the function of a wide range of viral envelope glycoproteins, thereby blocking virus infection. These include the IFITM proteins, guanylate binding protein 5, the SERINC proteins, and the membrane-associated RING-CH (MARCH) family of RING-finger E3 ubiquitin ligases. MARCH proteins downregulate cell-surface proteins involved in adaptive immunity. The RING-CH domain of MARCH proteins is hypothesized to ubiquitinate the cytoplasmic tails (CTs) of target proteins leading to their proteasomal or lysosomal degradation. Three MARCH proteins (MARCH1, 2, and 8) have recently been reported to target the HIV-1 envelope glycoprotein (Env) and vesicular stomatitis virus G glycoprotein (VSV-G), thereby impairing the infectivity of HIV-1 virions bearing these glycoproteins. We show that MARCH protein expression is rapidly induced by interferon (IFN) treatment and that the antiviral activity of MARCH proteins extends to the Ebola virus glycoprotein (EboV-GP) and the SARS-CoV-2 spike (S) protein. We observe that the MARCH protein targeting of VSV-G is to a large extent CT-dependent. In contrast, MARCH1 targeting of HIV-1 Env, EboV-GP, and SARS-CoV-2 S protein does not require the CT, indicating that MARCH-mediated inhibition of these viral glycoproteins is likely indirect and via targeting a cellular host factor(s) involved in glycoprotein trafficking. Confocal microscopy data demonstrate that MARCH proteins are able to trap the viral glycoproteins in an intracellular compartment and bearing lysosomal markers. These results clarify the mechanism by which MARCH proteins antigenize viral glycoproteins and provide insights into the antiviral role of cellular inhibitory factors in Env biogenesis, trafficking, and virion incorporation.

26 IMMUNE RESPONSES TO SARS-CoV-2
Akiko Iwasaki, Yale University, New Haven, CT, USA
The clinical presentation of COVID-19 involves a broad range of symptoms and disease trajectories. Understanding the nature of the immune response that leads to recovery over severe disease is key to developing effective treatments for COVID-19. In this talk, I will discuss immune responses in COVID-19 patients with moderate and severe disease. I will compare viral load, immune phenotype, and cytokines that are predictive of mortality and discuss signatures of cytokines and growth factors that associate with recovery vs disease exacerbation. I will also discuss sex differences in immunity to SARS-CoV-2 and how such differences correspond to disease outcomes.

27 THE POTENTIAL OF LONG-ACTING INJECTABLE DRUGS FOR PREVENTION AND TREATMENT OF TB
Eric Nuermberger, The Johns Hopkins University, Baltimore, MD, USA
More effective and readily implementable preventive therapy for tuberculosis (TB) is a critical unmet need in order to end the TB epidemic. Long-acting injectable formulations of TB drugs have great potential to meet this need, especially in pregnant individuals and children. This presentation will describe how long-acting injectable TB drugs could address important obstacles in the prevention and treatment of tuberculosis, illustrate an example where proof-of-concept has been achieved, and discuss future directions and challenges for further development.

28 PREGNANCY, HIV, AND TUBERCULOSIS: CURRENT PRACTICES AND RESEARCH OPPORTUNITIES
Jyoti S. Mathad, Weill Cornell Medicine, New York, NY, USA
Tuberculosis is a leading cause of maternal mortality, especially among women living with HIV. Women are most likely to develop active tuberculosis during and immediately after pregnancy. But the prevention and management of tuberculosis during pregnancy remain contentious, and national guidelines vary widely owing to insufficient data. This presentation will review: (1) the global burden of tuberculosis in women of reproductive age; (2) how pregnancy and the postpartum period affect M. tuberculosis immunology in women with and without HIV; (3) how to screen and treat pregnant women for tuberculosis infection, including data on isoniazid preventive therapy, rifapentine-containing regimens, and potential drug–drug interactions with antiretrovirals; (4) how to diagnose and treat pregnant women for tuberculosis disease, including drug-resistant tuberculosis; and (5) high-priority research areas for tuberculosis research in pregnant and postpartum women.
29 ADVANCES IN PEDIATRIC TUBERCULOSIS PREVENTION AND TREATMENT

Nicoletta Salazar-Austin, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

The overlapping HIV and tuberculosis (TB) epidemics have been devastating for children and adolescents living with HIV. This group continues to suffer disproportionately high TB-associated mortality. Poorly implemented TB prevention programming, limited sensitivity and specificity of diagnosis tools, and interactions with antiretroviral and anti-TB drugs continue to plague prevention and management of TB/HIV co-infection in children and adolescents living with HIV. This presentation will discuss recent advances and continued challenges in pediatric TB diagnosis, treatment, and prevention for both children and adolescents living with HIV and HIV-exposed uninfected (HEU) children.

30 IMPACT OF COVID-19 PANDEMIC ON HIV AND TUBERCULOSIS: WHO PERSPECTIVE

Soumya Swaminathan, World Health Organization, Geneva, Switzerland

The COVID-19 pandemic has infected more than 100 million people, killed more than 2.4 million, and had a major impact on the health system’s ability to deliver essential health services. The impact of COVID-19 on other infectious diseases such as HIV and tuberculosis (TB) has been immense, particularly in low-resource settings with high HIV and TB burden. Ongoing TB data collection and analysis from 200 countries have shown reduced access to care in outpatient and inpatient facilities, impacting the entire care cascade, including prevention, with case detection rates dropping by over 50% in some endemic countries in 2020. By its negative impact on poverty and malnutrition, it is possible that TB incidence could actually increase, strengthening the argument for robust prevention measures. The pandemic has caused significant disruption to HIV programs by limiting access to life-sparing antiretrovirals due to movement restrictions, local stockouts, and decrease in uptake of facility-based services. These disruptions are also expected to have reverberated some of the progress made in preventing vertical transmission of HIV, resulting in increased numbers of paediatric HIV infections. Therefore, strengthening systems for the maintenance of HIV, TB/HIV, and TB services is an urgent need in many high-burden countries. Although COVID-19 has challenged TB and HIV programmes, it has also offered several lessons, including how we join forces, innovate, and accelerate research and development. Some examples are the use of digital tools for contact tracing, use of AI-based diagnostic algorithms, widespread sharing of genomic sequence data to track virus evolution and emergence of new variants, and large multisite clinical trials to test new therapeutics and vaccines. The development and evaluation of new TB and HIV treatments and vaccines should learn from the past year of accelerated development and explore new models of public-private partnership for the development of global public goods. Despite progress, vulnerable populations such as children and pregnant women continue to lag behind innovations for TB and HIV, and these groups need to be included in clinical trials much sooner. Finally, we need to expand and strengthen the integration of services within the primary healthcare platform, optimizing differentiated service delivery, community engagement and the use of digital technologies to reach those most at risk of TB and HIV with screening, prevention, diagnosis, and treatment.

31 DOES HIV IMPACT COVID-19 SUSCEPTIBILITY OR SEVERITY?

Julia Del Amo, Ministry of Health, Madrid, Spain

In the early days of the COVID-19 pandemic, the HIV community became preoccupied that COVID-19 could be more severe in people with HIV (PWH) because of immunodeficiency, higher prevalence of comorbidities and immunosenescence, which had been associated with poorer COVID-19 outcomes. Nonetheless, HIV was not initially identified as disproportionally prevalent in hospitalized patients with COVID-19, an observation that still holds. It was also argued that because of immune dysfunction, PWH could be less likely to develop the harsh immunologic response that complicates COVID-19 and that some antiretroviral drugs could impair SARS-CoV-2 replication. Numerous studies have been published since, some with apparently contradictory conclusions. Different epidemiological designs and information sources have been used, from case reports to population-based cohorts. Some have compared PWH to people without HIV; others have made comparisons across PWH with different characteristics. Some have reported higher COVID-19 mortality in PWH compared with people without whereas others have not, but there are important differences in the comparison populations and confounders adjusted for. Associations between CD4 cell counts and COVID-19 outcomes are not consistent. The role of protease inhibitors on SARS-CoV-2 RNA-dependent RNA-polymerase has been ruled out, but evidence of effects of tenofovir disoproxil fumarate is accumulating from observational studies. Some of the well-established risk factors for SARS-CoV-2 acquisition—high mobility and social interaction, belonging to racial and ethnic minorities and socially disadvantaged groups—are more common in PWH than in people without. Some of the risk factors for COVID-19 severity—hypertension, diabetes mellitus, chronic obstructive pulmonary disease, renal disease and cancer—are also more common in PWH than in populations without HIV of similar age and sex. This presentation will summarize the state of the art and will highlight the importance of the choice of the groups against which COVID-19 outcomes in PWH are compared. Finally, it will also address that in order to establish the effect of HIV on SARS-CoV-2 susceptibility and COVID-19 severity, it is necessary to control for confounding variables that may be partly or fully responsible for the reported associations. It is also important to understand the role of comorbidities that are more common in PWH and may be the result of HIV infection.

32 THE IMPACT OF COVID-19 ON THE HIV PANDEMIC WORLDWIDE

Andrew D. Kambugu, Makerere University, Kampala, Uganda

With the emergence of the COVID-19, the loss of the hard-won momentum towards HIV epidemic control has become a major concern globally. The containment measures associated with COVID-19, including lockdowns, travel restrictions and physical distancing, which result in restricted access to essential services, compounded by the diversion of human and other key resources to address the pandemic, have led to significant disruptions in HIV service delivery and demand. During the earlier phase of the COVID-19 outbreak, modeling studies estimated a 10% increase in deaths among persons living with HIV in low- and middle-income countries (LMICs) as a result of the health systems disruptions occasioned by the pandemic, with interruptions in antiretroviral supplies being the key driver of mortality. Emerging data from multi-country surveys indicate that almost 1 in 7 countries have had either high or very high disruptions in HIV service delivery. Specifically, COVID-19 has resulted in substantial disruptions in HIV testing services, resulting in significant reductions in HIV case identification and treatment initiation across many age groups. There is also emerging evidence of reductions in patient retention and viral suppression. This talk will focus on the impact of COVID-19 on the HIV pandemic worldwide. It will highlight the drivers of the disruptions to the HIV care system, from containment measures including lockdowns and other restrictions, the diversion of substantial resources to address COVID-19, the high demand on the health systems, to COVID-19 stigma and other human rights concerns. The data suggest that the most vulnerable communities are bearing the brunt of the COVID-19-induced health systems disruptions. The talk will summarize insights from local and regional modeling studies on the impact of COVID-19 on HIV health systems as well as share emerging data on the direct and indirect impacts on services delivery across different regions. Finally, the talk will highlight proposals for and documented approaches to mitigating the effect of COVID-19 on the HIV pandemic.

33 THE IMPACT OF COVID-19 BEYOND HIV

Helen Bygrave, International AIDS Society, Geneva, Switzerland

The impact of the COVID-19 pandemic spans across our health systems. Whilst mortality due to COVID-19 itself dominates headlines, it will only be upon reflection of all-cause mortality that the full impact of COVID on death will be known. Globally, 90% of countries surveyed by the World Health Organization (WHO) in 2020 experienced disruptions in health services, with the greatest disruptions being reported in low- and middle-income countries (LMICs). COVID-19 has exposed what those of us working in HIV have long understood — social inequities and weak health systems are only exacerbated by a health emergency. There is increasing acknowledgment that HIV services need to be person-centred and ensure integration of other essential health services, such as vaccination for HIV-exposed and HIV-infected infants, contraceptive care and services for other chronic non communicable diseases (NCDs). However, these services have been some of the most frequently disrupted during the pandemic. A study commissioned by UNICEF estimated that an additional 6,000 children could die every day from preventable causes as the COVID-19 pandemic continues to weaken health systems and disrupt routine services.
estimates demonstrate that a 10% proportional decline in use of short- and long-acting reversible contraceptive methods in LMICs would result in an additional 49 million women with unmet need for modern contraceptives and an additional 15 million unintended pregnancies over the course of 1 year. Globally, those living with NCDs have an increased risk of severe disease if infected with COVID-19 but in over half of countries surveyed with community transmission of COVID-19, hypertension, diabetes, and cancer services were significantly disrupted. This presentation will present the global data on how health systems have been disrupted by the COVID-19 pandemic and, through the lens of vaccination, contraceptive care, and NCDs, describe not only the impacts, but how health services have responded and adapted. Learning from these adaptations, many of which have overcome long-term policy barriers, we must take the opportunity to build our health systems back better as we plan our recovery.

34 FEAR AND COVID-19: EXPERIENCES ON THE GROUND

Francois Venter, Ezintsha, Wits Reproductive Health & HIV Institute, Johannesburg, South Africa

The impact on services and supply lines of the epidemic and various forms of lockdown is covered in preceding presentations. I will focus more on the personal impact of the COVID-19 pandemic on HIV-positive patients, families, and health workers. There is little research on social impacts, especially from resource-poor environments, so this relies somewhat on personal experience, colleague and patient anecdote, and (often fragmented) media reports, largely from the Southern African region. Reports from patients, and substantiated by informal donor monitoring, suggest large numbers of patients endured fear-driven antiretroviral interruptions, conservatively estimated at over a million patients (around 20%) in South Africa, possibly worse in countries with weaker supply systems. HIV testing, antiretroviral initiations, and male circumcision programmes ceased to operate for much of 2020. Fear was voiced by patients and families on several fronts: Many countries used iron-fisted security approaches (and used to quash political opposition, as happened in Uganda), with over 340 000 people arrested in South Africa in 2020 alone for lockdown offences, with footage of soldiers brutally forcing people off streets into crowded shacks. Many patients reported fear of being arrested while collecting their medication. Limited and crowded public transport, as well as distrust of health services, contributed to the perception of lockdown as a threat to health. This personal experience of fear by patients (and in some cases families) on top of the general societal experience of fear—evident in some parts of the world, and reminiscent of HIV transmission concerns in the very early 80s. There is some evidence that health systems and health workers demonstrated remarkable resilience in subsequent “2nd waves” as experience with protection strategies. In the past decade, a number of HIV-1 bNAbs have been developed and are undergoing clinical evaluation. We will discuss results from both preclinical and clinical studies of anti-HIV-1 bNAbs and their potential role in HIV prevention, therapy, and cure strategies.

37 SUSTAINED DELIVERY AND LONG-ACTING AGENTS FOR PREVENTION OF HIV

Linda-Gail Bekker, University of Cape Town, Cape Town, South Africa

The approval, availability, and scale-up of oral preexposure prophylaxis (PrEP) for HIV continues to impact on population level HIV incidence around the world, shining a spotlight on the potential of primary prevention in ending the HIV epidemic. Yet, for many reasons daily oral PrEP is not and may never be feasible for every individual and every setting. Long-acting agents, long a key focus of research and development, are finally coming into their own and getting ready to offer PrEP consumers an expanded choice of options. This talk will provide an overview of the menu of long-acting agents becoming available, their efficacy and safety profiles, their advantages and limitations, and how we can prepare to mitigate challenges.

38 HIV-1 AND SARS-CoV-2: DURABILITY OF HOST IMMUNE RESPONSES FROM VACCINATION OR INFECTION

This highly interactive session begins with a brief overview of the issue or controversy. The scientific experts each offer their opinions or observations in a 5-minute summary, followed by a 20-minute, spirited discussion among the panel members. The moderator will bring in comments and questions from the audience. This session will address the waning immunity in SARS-CoV-2 infected populations, T-Cell immunity in SARS-CoV-2, population-level immunity, programming durable immunity, and pathological B-cell activation during SARS-CoV-2 infection.

39 FROM DAILY PILLS TO MONTHLY SHOTS FOR HIV PREVENTION AND TREATMENT: CAN EFFICACY BE TRANSLATED INTO EFFECTIVENESS?

This panel discussion will address the challenges of long-acting agents for HIV prevention and treatment at the clinic and population levels. The panel will present insights into the use of long-acting agents for treatment and prevention, the latest information on pharmacokinetic and drug resistance considerations with long-acting agents, and perspectives on implementation considerations from the US and global physicians.

40 PrEP SCALE-UP TO MEET UNAIDS 2030 GOAL: IT JUST ISN’T POSSIBLE—OR IS IT?

This interactive discussion will highlight the challenges that need to be overcome to achieve the 2030 UNAIDS targets. Opportunities and challenges to meeting these goals via a singular focus on antiretroviral treatment for people with HIV (ie, absent biomedical prevention scale-up) or in partnership with biomedical prevention will be highlighted, using recent randomized clinical trial data on treatment as prevention (TasP) programs and recent programmatic accomplishments in pre-exposure prophylaxis (PrEP) scale-up as starting points for discussion.

41 INTRINSIC RESISTANCE OF RESERVOIR CELLS TO IMMUNE KILLING

R. Brad Jones, Weill Cornell Medicine, New York, NY, USA

Cytotoxic T-cell (CTL) responses against HIV play a critical role in partially controlling viral replication in the absence of antiretroviral therapy (ART), but fail to eradicate HIV reservoirs. The ability of these reservoirs to persist on ART, despite CTL, is generally attributed to three primary factors: 1) viral latency—a barrier to detection by CTL, 2) inadequate magnitudes or effector functions of CTL, and 3) sequestration of some components of the reservoir in anatomical sanctuaries that largely exclude CTL. Here, we present evidence, rationale, and mechanistic insights supporting the existence of a fourth element, arising from differences in the intrinsic sensitivities of target cells to elimination by CTL. Target cells play active roles in the process of CTL killing, through participation in the formation of immunological synapses, and by “deciding” whether or not to undergo apoptosis, based on the integration of complex signaling pathways (which include negative regulators, eg, Serpin B9, BCL-2). The HIV reservoir comprises populations of long-lived infected cells that can undergo clonal expansion. The properties of these cells, and their progeny, thus become a key factor in understanding and overcoming HIV persistence. We will summarize the evidence supporting that intrinsic resistance to CTL is a property of reservoir-harboring cells on long-term ART, which may have limited the efficacy of “kick
and kill strategies to date, as well as the role of the pro survival protein BCL-2 in this phenomenon, and opportunities to overcome this therapeutically. In addition to BCL-2, we will present an update on additional diverse mechanisms of CTL resistance emerging from transcriptional screens, and in vitro functional validation and investigation, presenting results derived from both CD4+ T-cell and macrophage targets. These results will be contextualized alongside the relatively well-developed understanding of mechanisms at play in certain types of cancer, where the clonal expansion of tumor cells enables selection for CTL-resistant clones. In emphasizing the role of reservoir-harboring CTL targets, not only as presenters of antigen, but also as active and self-regulating partners in the processes of cytolysis, we hope to highlight a rich source of potential therapeutic targets that may augment strategies aimed at depleting HIV reservoirs.

42 HOW TO GENERATE GOOD KILLERS BY INITIATING ART (NOT TOO) EARLY?
Lydie Trautmann, Oregon Health and Sciences University, Portland, OR, USA
HIV infection results in significant defects in immune cell functions, including the inability to kill HIV infected cells due to chronic antigen exposure and immunosuppressive mechanisms triggered by chronic infection and inflammation. These immune dysfunctions are incompletely resolved by ART, resulting in viral reservoir persistence that impedes efforts to achieve long-term HIV remission. Two main immune cell types exhibiting cytolytic activity are necessary to eliminate HIV-infected cells in the body: CD8 T cells and NK cells. However, HIV infection renders both CD8 and NK cells dysfunctional, even after long-term ART. Some mechanisms of dysfunction in these cells will be discussed, as well as the effect of latency reversing agents on the function of these cells. Since these cells play a critical role in limiting viral replication early in acute HIV/SIV infection, early initiation of ART has been hypothesized to help preserve their capacity to efficiently eliminate HIV infected cells. Very limited data are available on the effect of early treatment on NK cell function. For CD8 T cells, while early initiation of ART in the first weeks of infection leads to the generation of cells that proliferate more and are better killers than when ART is initiated in chronic infection. However, these cells are at lower numbers in individuals who initiated treatment early. New data from the NHP model show that CD8 T cells with preserved function after early ART initiation can help reduce the viral set point, but their response is still too late to control viral rebound after ART cessation. Therefore, early initiation of ART can generate good killers but the timing of expansion and differentiation of these cells is still a barrier for HIV control. In addition, very early ART initiation leads to even smaller numbers of HIV-specific CD8 T cells and prevents development of antibodies that promote NK cell-mediated antibody-dependent cellular cytotoxicity, suggesting that timing of ART initiation in acute infection is important. For people who have not been treated early enough, immune checkpoint blockers are being tested to determine if they are able correct the dysfunctions in killing as has been shown in cancer. The use of therapeutic vaccines and administration of genetically engineered cells are also being explored to boost these cytotoxic responses. These strategies aiming at boosting CD8 T cell and NK cell cytolytic functions and induce HIV remission will be presented.

43 BREAKING THE B-CELL FOLLICULAR BARRIER TO ELIMINATE VIRAL RESERVOIRS
Afan Okoye, Oregon Health and Sciences University, Portland, OR, USA
Follicular helper T cells (TFH) are a specialized subset of CD4+ T cells that interact with antigen-specific B cells within specialized structures known as germinal centers in B cell follicles of secondary lymphoid tissues. This interaction is required for antibody affinity maturation and the differentiation of germinal center B cells into long-lived memory B cells and plasma cells. Migration into B-cell follicles is highly regulated by chemokine/chemokine receptor interactions, in particular CXCL13/CXCR5, providing for specific ingress of CXCR5+, CD4+ TFH and other minor populations of CXCR5+ regulatory T cells, but not other (CXCR5-) T cell types, including the vast majority of antiviral effector CD8+ T cells. CD4+ TFH are susceptible to HIV (and SIV) infection and can support active viral replication and production in vitro and in vivo. Untreated HIV/SIV infection leads to an accumulation of CD4+ TFH, which can contain relatively high proportions of HIV/SIV RNA+ cells. In SIV-infected monkeys, productive SIV infection becomes almost exclusively CD4+ TFH cell-restricted upon attainment of elite virologic control, suggesting that the highly effective antiviral CD8+ T-cell responses in SIV elite controllers were able to almost completely clear and/or suppress productive SIV infection in extra-follicular T-cell zones, but not within B cell follicles. This is highly relevant for HIV cure because it implies even the most effective antiviral CD8+ T-cell responses will be limited in their ability to destroy or suppress reactivating virus in CD4+ TFH both during ART and after ART cessation. This talk will discuss ongoing efforts to disrupt this “B-cell follicular sanctuary” to fully evaluate the ability of virus-specific CD8+ T-cells to achieve durable, post-ART virus remission.

44 TYPE 1 INTERFERON RESISTANCE OF REBOUND HIV-1
Beatrice H. Hahn, University of Pennsylvania, Philadelphia, PA, USA
Type 1 interferons (IFN-I) are potent innate antiviral effectors. However, harnessing these cytokines for HIV-1 prevention, treatment, and cure strategies has been hampered by an incomplete understanding of the role of endogenously produced IFN-I in viral control. To examine the kinetics of the IFN-I response over the course of HIV-1 infection, we generated 500 clonally-derived HIV isolates from the plasma and CD4+ T cells of 26 individuals sampled prospectively before and after antiretroviral therapy (ART), and/or during analytical treatment interruption (ATI). Determining the concentration of IFNα2 and IFNβ that reduced viral replication in vitro by 50% (IC50) we found consistent changes in the sensitivity of HIV-1 to IFN-I inhibition both across individuals and over time. IFN-I resistance was uniformly high during acute infection, decreased in all individuals in the first year post-infection, was reacquired concomitant with CD4+ T cell loss, and remained elevated in individuals with accelerated disease. Isolates obtained by viral outgrowth during suppressive ART were relatively IFN-I sensitive, resembling viruses circulating just prior to ART initiation. However, viruses that rebound following treatment interruption displayed the highest degree of IFNα2 and IFNβ resistance observed at any time during the infection course. Interestingly, IFNα2 IC50 values of rebound isolates from individuals treated with pegylated IFNα2 before and during ART interruption were on average 1.8-fold higher than those of rebound isolates from IFNα2 untreated individuals, and such differences were not observed for the corresponding IFNβ IC50 values. IFN-I-resistant viruses did not share a particular biological phenotype, but some rebound viruses were exquisitely macrophage tropic. Finally, an analysis of post-ATI isolates generated by viral outgrowth suggested that treatment interruption may have re-seeded the reservoir with IFN-I resistant viruses in some patients. These findings indicate a dynamic interplay between host innate immune responses and the evolving HIV-1 quasispecies, with the relative contribution of IFN-I to HIV-1 control impacted by both ART and analytical treatment interruption. Although elevated at transmission, host innate pressures are the highest during viral rebound, limiting the viruses that successfully reactivate from latency to those that are IFN-I resistant.

45 TRIPLE-DRUG ART, DUAL ART, OR JUST ART?
Jose R. Arribas, Hospital La Paz Institute for Health Research, Madrid, Spain
Over the last 2 years, for the first time in the history of modern antiretroviral therapy (ART), expert guidelines (EACS, DHHS, IAS–USA) have started to include 2-drug ART regimens as recommended combinations for most people with HIV (PWH), both as initial and as maintenance therapy in the setting of virologic suppression. Currently 2-drug ART can be administered orally and very soon it would be available for prescription as long-acting formulations. This advance has been possible because of the advent of second-generation integrase strand transfer inhibitors with a genetic barrier so high they require only 1 reverse transcriptase inhibitor to complete an effective ART regimen. In this presentation I will review the efficacy and safety data supporting the use of two-drug oral ART in ART-naïve and virologically suppressed PWH and of long-acting two-drug ART in virologically suppressed PWH. I will also discuss current gaps of knowledge and the pros and cons of using 2- versus 3-drug ART regimens. I will also review scenarios that are more suitable for two-drug ART, in both oral and long-acting forms.

46 NOVEL ANTIRETROVIRAL THERAPIES IN CLINICAL DEVELOPMENT
Alexandra L. Calmy, University Hospitals of Geneva, Geneva, Switzerland
Innovative drugs in the development pipeline for HIV treatment are at a crossroads. The model of introducing drugs with incremental improvements within existing drug classes is now fading. Drugs aimed at novel targets may have a greater impact, especially for providing new options in individuals multi-exposed to ARVs. The scientific community has moved research into combined strategies aiming at long-acting molecules developed with multifaceted objectives way beyond the sole control of viral suppression. Novel HIV drugs and combinations are being developed for prevention, treatment, and/or attacking the reservoirs of latent HIV. Continuous investment in novel HIV treatment
strategies should be ensured, despite competing priorities, in order to improve the quality of life of affected individuals and to achieve the goal of epidemic control. Public health improvement strategies will require that early on in the development of drugs, diversity is represented at all stages of clinical research.

**47 ARVs AND ART FOR NEWBORNS AND INFANTS: A LAST FRONTIER**

Mohemdran Archarya, University of KwaZulu-Natal, Durban, South Africa

This state-of-the-art talk on antiretroviral drugs and antiretroviral treatment in neonates and infants will focus on: 1) the considerations when evaluating the safety and dosing of new ARVs in infants and young children, 2) the additional considerations when treating premature infants with antiretroviral drugs for prophylaxis or treatment, 3) the evolving paradigm around the use of antiretroviral drugs for prevention and treatment of HIV during the newborn period, 4) the role of transplantational transfer of antiretroviral drugs in loading the neonate in the peripartum period, 5) discussion on currently available antiretroviral drugs used to treat neonates/infants, and 6) discussion on antiretroviral drugs and delivery systems that are in the pipeline for prevention and treatment of HIV across the pediatric age range.

**48 GOT ANYTHING FOR THIS COUGH? NEW ANTIVIRALS FOR TREATMENT OF SARS-CoV-2**

Davey M. Smith, University of California San Diego, San Diego, CA, USA

Attendees to this presentation will be introduced to currently available and promising antiviral agents for SARS-CoV-2. These agents will include monoclonal and polyclonal antibodies, protease inhibitors, interferon-based agents, and small molecule drugs. The presentation will also touch on how viral variants of concern may impact the activity of some of these agents.

**49 SINGLE VASCULAR CELL HETEROGEOEITY IN HEALTH AND DISEASE: A COVID-19 UPDATE**

Peter Carmeliet, University Hospitals Leuven, Leuven, Belgium

On the basis of emerging evidence from patients with coronavirus disease 2019 (COVID-19), we postulate that endothelial cells are essential contributors to the initiation and propagation of severe COVID-19. COVID-19, caused by the betacoronavirus SARS-CoV-2, is a worldwide challenge for health care systems. The leading cause of mortality in patients with COVID-19 is hypoxic respiratory failure from acute respiratory distress syndrome (ARDS). To date, pulmonary endothelial cells (ECs) have been largely overlooked as a therapeutic target in COVID-19, yet emerging evidence suggests that these cells contribute to the initiation and propagation of ARDS by altering vessel barrier integrity, promoting a procoagulative state, inducing vascular inflammation (endotheliitis) and mediating inflammatory cell infiltration. Therefore, a better mechanistic understanding of the vasculature is of utmost importance. Here, we discuss current insights into endothelial cell biology in health and disease focusing on their heterogeneity between and within vascular beds, the divergent global and metabolic characteristics they display and that correlate with the specific functions of the particular endothelial cell subtypes, and discuss the link between endothelial cells, viral infection, and inflammatory changes, proposing novel therapeutic strategies.

**50 BODY ON FIRE: MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN**

Elizabeth A. Whittaker, Imperial College London, London, UK

In April 2020, reports of an inflammatory condition with overlapping features of Kawasaki disease and toxic shock syndrome emerged in Italy and the UK, and subsequently other countries in Europe, the Americas, and Asia have reported cases of this rare syndrome, now called Paediatric Inflammatory Multisystem Syndrome (PIMS-TS) or Multisystem Inflammatory Syndrome in Children (MIS-C), that is temporally associated with SARS-CoV-2 infection. Case definitions use criteria including clinical manifestations (fever, inflammation, organ dysfunction), elevated biochemical markers of inflammation, and evidence of contact or infection with SARS-CoV-2, with exclusion of another microbial cause. There are many questions currently emerging that need to be answered—for example, how the pathophysiology of MIS-C differs from other paediatric conditions such as Kawasaki disease, Kawasaki disease shock syndrome, and toxic shock syndrome. Does the immunology differ from that of severe COVID-19 disease in adults? What are the optimal treatment regimens? Can we improve diagnosis, and hence early recognition and outcomes? In this talk, I will outline the epidemiology, clinical presentation and management, as well as our current understanding of this emerging inflammatory condition.

**51 THINK ABOUT IT: NEUROLOGIC MANIFESTATIONS OF COVID-19**

Benedict D. Michael, University of Liverpool, Liverpool, UK

In this presentation, Dr Michael will review the results of the clinical and epidemiologic studies of the neurological and neuropsychiatric complications associated with COVID-19, their implications for clinical practice, and insights from on-going studies of underlying disease mechanisms.

**52 COVID-19: CONCERNS OF THE HEART**

Valentina O. Punthmann, Goethe University, Frankfurt, Germany

Cardiac involvement due to COVID-19 infection is an increasingly recognized complication. In this talk, Dr Punthmann will provide an overview of the current state of the knowledge. She will address some of the controversies in terms of the diagnostic choices in detecting cardiac inflammation. She will also share the insights from the Frankfurt Cohort, the largest prospective study of patients with recent COVID-19 infection and serial follow-ups with cardiovascular magnetic resonance.

**53 YOUNG WOMEN: ADHERENCE, EFFECT, AND RETENTION**

Thesla Palanee-Phillips, Wits Reproductive Health and HIV Institute, Johannesburg, South Africa

Biomedical HIV prevention strategies such as preexposure prophylaxis (PrEP) are designed to maximize public health impact at a population level. Despite current HIV prevention and treatment tools having helped reduce incident HIV infections and AIDS-related deaths in the last decade, urgency remains around the need to identify additional options. Meeting global HIV reduction targets will require improved service-delivery platforms to get prevention choices to people at risk at an individual level while layering prevention coverage to achieve population-level impact. HIV prevention programmes usually focus on prevention of HIV through a complementary combination of behavioural, biomedical and structural strategies. Scientific evidence of oral PrEP efficacy as a strong HIV prevention tool has gained strength from data from demonstration projects. Daily oral PrEP is currently the only available prevention product for women with regulatory approval, other than condoms, although the monthly dapivirine vaginal ring (DVR) received a positive scientific opinion from the European Medicines Agency (EMA) in July 2020 and a WHO recommendation in January 2021, and may become available in some countries soon. Despite increasing availability of oral PrEP and the anticipated future availability of the DVR option, both products have demonstrated varying levels of effectiveness in young women. Low adherence has contributed to a lack of efficacy in clinical trials and, coupled with high rates of PrEP discontinuations observed in clinical practice, threatens their public health impact. These outcomes support pursuit of a multi-pronged approach: continued and development of highly effective, affordable, discrete HIV prevention interventions that are acceptable to end-users while ensuring higher levels of protection, all while intensifying efforts to better understand barriers and facilitators to oral and topical PrEP initiation, execution and persistence for protection. If strategies to circumvent known barriers to adherence to PrEP use can be identified and implemented, more effective use of current as well as future PrEP options may be assured through their extension. This presentation will focus on understanding challenges related to adherence, retention, and their impact on PrEP efficacy among women and explore mechanisms to strengthen the HIV prevention toolbox arsenal.

**54 CHALLENGES AND OPPORTUNITIES IN IMPLEMENTING PrEP IN KEY POPULATIONS**

Gregorio A. Millett, amfAR, New York, NY, USA

Greg Millett will discuss PrEP in key populations, including men who have sex with men, transgender individuals, sex workers, incarcerated populations, and refugees. His talk will focus on implementation issues on the ground in key populations across the planet, highlighting both challenges and models of success.

**55 MODELS OF DELIVERING PrEP IN EAST AFRICA: INNOVATIONS AND LESSONS LEARNED**

Moses R. Kamya, Makerere University College of Health Sciences, Kampala, Uganda

Oral pre-exposure prophylaxis (PrEP) is highly effective and could bring us closer to HIV elimination targets if offered alongside testing and treatment. However, this effective HIV prevention intervention is only beginning to be scaled up in many settings globally. A number of demonstration projects and
implementation research studies have been conducted in East Africa among populations with increased potential for HIV exposure. Implementation experiences are beginning to emerge, including high levels of interest in and willingness to use PrEP, but modest rates of persistence in some settings. In this presentation, we will discuss lessons learned regarding PrEP delivery from the first generation of demonstration and implementation studies in East Africa. We will explore models of facility and community-based PrEP delivery, associated PrEP uptake, use, and implementation experiences, both among subpopulations and the general population. We will also discuss future innovations in PrEP delivery, including strategies for achieving lower-barrier access and simplifying PrEP delivery. The implications of these findings for delivery approaches for oral PrEP and future long-acting prevention modalities will be highlighted.

**56 INCORPORATING INJECTABLE PrEP AND NEWER FORMULATIONS INTO LMIC SETTINGS**

Yogan Pillay, Clinton Health Access Initiative, Pretoria, South Africa

Dr Pillay will discuss perspectives about the rationale and considerations for long-acting PrEP as an additional HIV prevention tool for low and middle income countries, in addition to anticipating and preparing for implementation issues.

**57 ELITE CONTROLLERS: A MODEL FOR A FUNCTIONAL CURE OF HIV-1 INFECTION**

Xu Yu, Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA

HIV integrates into the host genome to establish life-long infection, requiring indefinite antiretroviral therapy. The development of novel strategies that induce a long-term, drug-free remission of HIV-1 infection has evolved as one of the highest-priority objectives for the HIV research community. Intriguingly, such a long-term, drug-free remission or “functional cure” of HIV-1 infection is naturally observed in a small number of HIV-1-infected individuals, termed “elite controllers” (ECs), who maintain undetectable levels of HIV-1 replication and do not show clinical evidence of HIV-1 disease progression. Recent advances in technology development allowed us to comprehensively profile the proviral landscape within HIV-1 reservoir cells in people living with HIV (PLWH). Near full-length individual proviral sequencing (FLIP-Seq) and matched integration site analysis (MIP-Seq) revealed that replication-competent intact proviruses accumulated in non-genic or heterochromatin regions of the human chromosomes in ECs. In extremely rare cases, intact proviruses were undetectable despite analyzing massive numbers of cells, suggesting a sterilizing cure of HIV-1 infection might have been achieved in these exceptional elite controllers. In addition, single-cell assays able to simultaneously capture the proviral sequences, the corresponding chromosomal integration sites, and HIV RNA transcriptional activities in unmanipulated patient-derived cells have recently been developed. This multidimensional analysis platform demonstrated that proviruses integrated into non-genic or heterochromatin regions of the human chromosomes had limited transcriptional activities and were in deep latency. Novel epigenomic profiling technologies will provide additional insights into the rebound-competent reservoirs. Assays that evaluate the qualitative, rather than quantitative, features of viral reservoir cells will be helpful for investigating the efficacy of clinical interventions aiming at a functional cure of HIV-1 infection.

**58 HIV TREATMENT IN PREGNANCY: BEYOND PREVENTION OF VERTICAL TRANSMISSION**

Shahin Lockman, Brigham and Women’s Hospital, Boston, MA, USA

Women comprise more than half of persons living with HIV globally, and most women with HIV will be pregnant at least once. It is essential to understand the impact of HIV treatment during pregnancy on not just vertical transmission but also on obstetric and maternal and child health outcomes, in order to optimize the health of women and children through their life course. The global scale-up of antiretroviral treatment (ART) over the past 10 years has resulted in a continued decline in new pediatric HIV infections (although challenges remain). The past decade has also offered an increased understanding that the antiretroviral regimen in pregnancy can affect health outcomes other than vertical transmission, and that adverse outcomes associated with antiretrovirals in pregnancy can disrupt global ART programs, particularly for women. These realizations have led to an appreciation of the importance of timely and robust data on the safety and expected efficacy of HIV drugs used in pregnancy and during lactation. This talk will briefly touch upon the prevention of vertical transmission and will then review major findings regarding the relationship between maternal ART regimens in pregnancy, and pregnancy outcomes and maternal and child health outcomes. Current recommendations for antiretroviral use in pregnancy and lactation will be summarized. Important evidence gaps will be highlighted, and potential approaches for addressing these gaps through more timely research in pregnancy will be discussed.

**59 SARS-CoV-2 AND THE HOST IMMUNE RESPONSE: GOOD VS BAD IMMUNITY**

This highly interactive session begins with a brief overview of the issue or controversy. The scientific experts each offer their opinions or observations in a 5-minute summary, followed by a 30-minute, spirited discussion among the panel members. The moderator will bring in comments and questions from the audience. This session will address several aspects of immunity directed to SARS-CoV-2, including innate, humoral, and cellular immune responses.

**60 COVID-19 CLINICAL CONTROVERSIES**

Based on illustrative case vignettes, a panel of experts will have a moderated and lively exchange of what they consider to be optimal treatment strategies and what issues require our attention in terms of potential long-term health concerns once patients recover from the acute episode of illness.

**61 COVID-19 DISPARITIES: HOW CAN THEY HELP MOVE THE NEEDLE FORWARD?**

This session will summarize critical data on the SARS-CoV-2 pandemic among several populations that experience disproportionate burdens of morbidity and mortality due to COVID-19. Speakers will also give recommendations for key interventions that could substantially decrease these disparities and participate in an interactive panel discussion with audience participation.

**62 NOVEL FINDINGS OF NEURONAL MODULATION OF HIV EXPRESSION IN MICROGlia**

Jonathan Karn, Case Western Reserve University, Cleveland, OH, USA

Although ART dramatically lowers the levels of viral RNA in the brain, it does not reduce the incidence of HIV-associated neurocognitive disorders (HAND), which still develop in up to 50% of persons with HIV. Initial studies indicated paradoxically that the development of HAND correlates strongly with systemic inflammation and CNS inflammation, but did not correlate with the number of HIV-infected cells or viral antigens in the CNS. HIV latency was studied in a wide variety of systems, including immortalized human microglial cells, in IPS-derived microglial cells, in brain organoids modified to incorporate microglial cells, and finally, in humanized mice. Pathways regulating HIV transcription were studied by co-culture experiments, drug treatments, and ex vivo induction of HIV in latently infected cells. The identities and polarization states of the cells were demonstrated by single-cell RNA-seq studies. Entry of HIV into latency is in response to specific repressive signaling pathways, especially due to signals emanating from healthy neurons in co-culture experiments with IPS-derived neurons and in brain organoids. Although the mechanism underlying the neuronal silencing of HIV infections is not yet fully understood, key mediators of HIV silencing include the glucocorticoid (GR/NR3C1), Nurr1 (NR4A), and retinoid X (RXR) receptors, which can potentially be targeted by therapeutic drugs to prevent HIV reactivation. By contrast, reactivation of latent HIV occurs in response to inflammatory signals such as IL-1β, TNF-α or TLR agonists—all signals correlating with the severity of HAND. Humanized mice models developed by the Cannon and Karn laboratories show that human microglia recovered from the brains of the mice are indistinguishable from primary human microglia by surface markers and by single-cell RNA-seq (scRNA-Seq) analyses. We have combined these analyses with our highly sensitive next-generation sequencing assay for HIV transcripts, the EDITs assay, and demonstrated that human microglia isolated from HIV-infected mice carry latent HIV proviruses that can be reactivated ex vivo by TNF-α and poly (I:C). In vivo and ex vivo has demonstrated the linkage between inflammation and latent HIV infection of microglial cells. Building on detailed studies of the molecular mechanisms underlying HIV latency in microglial cells, we expect to identify and evaluate candidate therapies that interfere with these circuits and could serve as potential treatments for HAND.

**63 ROLE OF CNS CD4 T CELLS AND MACROPHAGES IN SIV NEUROPATHOGENESIS AND RESERVOIRS**

Vanessa Hirsch, National Institutes of Health, Bethesda, MD, USA

While the use of combination antiretroviral therapy effectively suppresses systemic viral replication, neurocognitive disorders remain a persistent clinical problem. Therefore, the use non-human primate models are necessary to...
study mechanisms of neuropathogenesis. Simian immunodeficiency virus (SIV)–infected non-human primates can serve as a relevant model for AIDS neuropathogenesis. The current SIV-induced encephalitis (SIVE)/neuroAIDS models are generally associated with rapid progression to neuroAIDS, which does not reflect the tempo of neuroAIDS progression in humans. In a recent study, we isolated a neuropathogenic clone SIVsm804E–CL757 (CL757) from an SIV-infected rhesus macaque. This virus causes more protracted progression to disease (approximately 1 year post-infection) and induces SIVE in 50% of inoculated animals, with high cerebral spinal fluid viral loads, multinucleated giant cells (MNGCs), glial nodules, and perivascular lymphocytic cuffing in the central nervous system (CNS). This latter finding is reminiscent of HIV encephalitis in humans but is not generally observed in rapid progressor animals with neuroAIDS. By isolating mononuclear cells from the brains of SIV-infected rhesus macaques with and without encephalitis, we show that immune cells invade the neuroparenchyma and increase in number in the CNS in animals with SIV-induced encephalitis (SIVE). Recently we studied which subsets of cells within the CNS were targeted by CL757 in animals with neurological symptoms. Immunohistochemistry of brain sections from these animals demonstrated infiltration of CD4+ T cells and macrophages to the sites of MNGCs. Moreover, an increase in mononuclear cells is isolated from the brain tissues of rhesus macaques (RM) with SIVE correlated with increased CSF viral load. Subset analysis showed a specific increase in brain CD4+ memory T cells (Br-mCD4), brain-macrophages, and Br-B cells. Both Br-mCD4s and Br-macrophages harbored replication-competent viral DNA as demonstrated by virus isolation by co-culture. However, only in animals exhibiting SIVE/neuroAIDS was virus isolated from Br-macrophages. These findings support the use of CL757 to study the pathogenesis of AIDS viruses in the central nervous system and indicate a previously unanticipated role of CD4 cells as a potential reservoir in the brain that might persist during antiretroviral therapy.

**64 MYELOID CNS HIV RESERVOIRS...THE DEBATE CONTINUES**

_Lishomna Ndlovu, Weill Cornell Medicine, New York, NY, USA_

Gallant efforts are ongoing to achieve sustained antiretroviral therapy (ART)–free HIV remission. With the exception of two stem-cell transplantation cases and the few instances of natural control, this has been proven challenging. Recent advances have highlighted the importance of myeloid reservoirs as sanctuaries of HIV persistence and therefore may partially be responsible for viral recrudescence following analytical antiretroviral treatment interruption. The ongoing debate in the field as to whether the central nervous system (CNS), encompassing the brain, spinal cord, and its cellular bodies, including myeloid cells, continues as to their contribution as sites of HIV persistence of latent replication or non-replication competent virus in the setting of ART. Further, most current human HIV cure–targeted clinical studies have primarily focused these efforts on targeting viral persistence in CD4 T cells in blood and tissue sanctuaries, and the lack of myeloid-centered CNS clinical trials focused on the CNS reservoir, either as primary or secondary endpoints, has hindered our understanding of the contribution of myeloid cells and the CNS as viral sanctuaries of rebound virus and may guide successes in future HIV eradication strategies. This presentation will highlight controversies, boundaries to overcome and new research efforts defining myeloid reservoirs and HIV persistence in the CNS and recent advances in HIV remission and cure trials that would be relevant in targeting this compartment, and make an argument as to their clinical relevance as we progress towards sustained ART-free HIV remission in all people with HIV.

**65 LESSONS FROM CNS HIV ESCAPE AND ATI FOR UNDERSTANDING HIV PERSISTENCE IN THE CNS**

_Sarah B. Joseph, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA_

Direct sampling of putative HIV-1 reservoirs in the brain parenchyma of participants on antiretroviral therapy (ART) is typically impossible, making cerebrospinal fluid (CSF) the only material available for querying reservoirs in the CNS of living participants. CSF collected from people on ART generally contains extremely low levels of HIV-1 RNA and CD4+ T cells. In contrast, elevated levels of HIV-1 RNA are observed in CSF collected from participants during CSF escape and treatment interruption (TI). Thus raising questions as to whether viral populations found in the CSF during CNS escape and TI represent virus production from reservoirs in the CNS or periphery. There is now sufficiently genetic and phenotypic data to indicate that HIV-1 populations in the CSF during CSF escape and TI have heterogenous origins with some emerging from myeloid reservoirs in the CNS and others arising from T cell reservoirs in the CNS and/or periphery. Cumulatively these results indicate that long-lived reservoirs can persist in CNS resident cells (i.e. microglia/macrophage), but the frequency of such reservoirs and their ability to generate viral recrudescence remains unknown.

**66 PREP DURING PREGNANCY AND BREASTFEEDING**

_John Kinuthia, University of Nairobi, Nairobi, Kenya_

High HIV incidence among pregnant and breastfeeding women in sub-Saharan Africa highlights the critical need for primary prevention efforts. Pre-exposure prophylaxis (PrEP) is an effective woman-controlled HIV prevention strategy that is safe for mother and infants. In 2017, Kenya rolled out PrEP implementation for individuals at substantial risk of HIV including pregnant and breastfeeding women. Maternal and child health (MCH) clinics provide an effective platform for PrEP delivery and are less stigmatized locations for women to access HIV preventive interventions. The PrEP implementation for Young Women and Adolescents (P-YHA) program was implemented in 16 facilities in Kisumu County, western Kenya in collaboration with the Kisumu County Department of Health and the National AIDS and STI control program to deliver PrEP to women particularly adolescent girls and young women attending MCH clinics in a high HIV prevalence region in Kenya. This talk will discuss lessons learnt implementing the P-YHA program including engagement with county and national government institutions, how PrEP was offered in MCH clinics, who took PrEP, reasons for initiating PrEP, reasons for not initiating PrEP among women with risk factors, and factors contributing to PrEP continuation.

**67 PREP ADHERENCE INTERVENTIONS FOR ADOLESCENTS AND YOUNG ADULTS**

_Sybil Hosek, Stroger Hospital, Chicago, IL, USA_

Oral pre-exposure prophylaxis (PrEP) is a remarkably effective means of HIV prevention when taken consistently. Young African women and young men who have sex with men (MSM) in the US have demonstrated lower levels of PrEP adherence than older adults across multiple open label trials and demonstration projects. Given the biological, behavioral, and social developmental factors that influence adherence among adolescents, it is critical to identify evidence-based adherence support interventions for PrEP use. Strategies are needed to optimize PrEP adherence among youth, particularly for a prevention behavior that requires repetition on a daily basis, such as oral PrEP use. This presentation will review key interventions that have shown success at improving PrEP adherence among adolescents as well as interventions currently being tested that may prove promising in the future.

**68 IMPROVING ADOLESCENT PREP USE: UNDERSTANDING DEVELOPMENTAL PROCESSES AND CONTEXT**

_Clau de A. Mellins, New York State Psychiatric Institute, New York, NY, USA_

Ending the global HIV epidemic will be stymied without concerted efforts focused on adolescents, who represented nearly 25% of all new HIV infections in 2019. PrEP has revolutionized adult prevention efforts, and its safety and acceptability has resulted in authorization for adolescents in the US since 2018, with increasing use globally. Yet, data to date suggest adolescent uptake and persistent adherence to PrEP, similar to treatment of HIV and other health conditions, is a challenge. The goal of this talk is to further understanding of the context and developmental processes that drive these challenges in order to help providers work more effectively with adolescents and inform interventions to improve PrEP uptake and adherence. Adolescence is marked by profound changes in physical, social, cognitive and emotional function that impact health behaviors. Adolescents are developing gender and sexual identities, adjusting to developmental tasks important to adult transition (e.g., greater independence from parents, decision-making autonomy, peer affiliations, community engagement), and initiating intimate relationships. Imaging studies have identified central nervous system changes through age 25 years that impact maturation of emotion regulation, cognition, problem-solving and decision-making skills. Although studies have focused on adolescence as a risky stage—associated with impulsivity, sensation-seeking, underdeveloped executive function, emotion dysregulation, experimentation with substance use and sexual behavior, and psychiatric disorders—for those at risk—adolescence also provides a critical period for developing lifelong healthy behaviors. The adolescent brain is designed for flexibility, tolerance of ambiguity, and targeted risk-taking and sensation-seeking—all key to adult transition. To optimize adolescent PrEP interventions, we advocate for (a) an appreciation of the neuro-
cognitive, emotional, and social strengths of this stage and (b) consideration of the multiple factors that foster or protect against behavioral health risks relevant to PrEP use (e.g., cognition, mental health; substance use; family, peer and provider relationships; HIV stigma; health care; cultural context). A more holistic and strengths-based approach to examining adolescent decision-making in the context of development, life circumstance, and future aspirations may lead to more innovative methods for promoting engagement in and persistent adherence to PrEP.

69 IMPROVING HOW WE MARKET PREP TO YOUTH
Elzette Rousseau, University of Cape Town, Cape Town, South Africa
The benefits of pre-exposure prophylaxis (PrEP) for HIV prevention are well-established, however efficacy in high-incidence locations will depend on whether young people can effectively access, use, and adhere to PrEP. Demand creation in acceptable spaces and with positive messaging not just about the benefits of PrEP but also about how PrEP can conveniently fit into young people’s lives are critical in motivating uptake and continued use. Young people, especially adolescent girls and young women (AGYW) in Africa, want ‘fast-PrEP’. This presentation will cover modalities of differentiated PrEP delivery convenient for young people as well as how to attract target population to these services. In addition, innovations (including mobile health) will be discussed as a way to engage young people, assist them in deciding if PrEP is for them, mitigate unintentional PrEP interruptions and facilitate prevention-effective adherence.

70 HEPATITIS C ELIMINATION: WHAT’S TAKING SO LONG?
Annie Luetkemeyer, University of California San Francisco, San Francisco, CA, USA
This talk will review current the status of the hepatitis C virus (HCV) epidemic in the US and globally, focusing on progress and impediments to meeting elimination goals. Dr Luetkemeyer will review models of successful widespread treatment and micro-elimination and strategies to address remaining barriers to elimination, including lessons learned from COVID-19 and approaches to highly impacted populations.

71 MECHANISMS AND TREATMENTS OF STEATOSIS IN HIV
Steven Grinspoon, Harvard Medical School, Boston, MA, USA
Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of disease, progressing from simple steatosis, through mixed steatosis and lobular inflammation, to nonalcoholic steatohepatitis (NASH), and ultimately advancing to more advanced fibrosis, cirrhosis, and end-stage liver disease. NAFLD is a manifestation of metabolic disease, associated with obesity, visceral adiposity, and insulin resistance, and contributes to an increased risk for cardiovascular disease. Increased flux to the liver of substrate, including fatty acids, increased intra-hepatic de novo lipogenesis and reduced hepatic fat oxidation, are major mechanistic factors. NASH is an increasing cause of liver transplants. Among people with HIV (PWH), specific pathogenic factors may relate to increased weight and hepatic toxicities, particularly with specific antiretrovirals, including integrase strand transfer inhibitors (INSTIs). In addition, PWH, may experience increased visceral adiposity, proinflammatory stimuli, immune activation, insulin resistance and stimulation of critical hepatic metabolic pathways by viral envelope proteins. The prevalence of NAFLD is high, estimated at 35%, and the phenotype exaggerated among PWH. For PWH, NAFLD is seen at a lower BMI in association with a higher prevalence of NASH in PWH. FDA approval for drugs in this class requires resolution of NASH without increasing fibrosis or vice versa. No drugs have yet to be approved for this indication in the general population, though many are now under investigation. Among PWH, a limited number of trials have been performed, but data do show some promising potential strategies. Therapeutic strategies that target steatosis, may work by reducing insulin resistance, (e.g., GLP-1 agonists and/or targeting de novo lipogenesis and hepatic fat oxidation [GHGR agonists]), or improving hepatic inflammatory, immune and fibrotic pathways (CCR2/5 antagonists). In addition, specific agents are being tested for effects on adipogenic/lipolytic pathways (PPAR gamma agonists, steroyl CO-A Desaturase Inhibitors), antifibrotic and anti-inflammatory properties. Overall, significant efforts are being made to develop successful strategies for NAFLD/NASH in PWH.

72 HARNESSING IMMUNITY TO CURE HBV
Ulrike Prozrer, Technical University of Munich, Munich, Germany
Currently, there is no curative treatment for hepatitis B virus (HBV) infection. Available antivirals control replication and mitigate inflammation but-as in HIV infection-cannot attack the nuclear persistence form of the virus. Virus-specific immunity can eliminate and control HBV, but antibody, as well as T-cell responses, are barely detectable in chronic infection. Activating HBV-specific immunity may therefore be the clue to achieve HBV cure.

73 NEW PROSPECTS FOR THE TREATMENT OF HBV
Heiner Wedemeyer, Medizinische Hochschule Hannover, Hannover, Germany
In this presentation, Heiner Wedemeyer will address new drug developments for hepatitis B management and cure. He will describe strategies that utilize current therapies to harness the immune response, discuss novel agents in the pipeline, and review how to best utilize these novel therapies in order to achieve functional cure with defined treatment endpoints in patients with chronic hepatitis B.

74 LESSONS LEARNED AND CHALLENGES IN COVID-19 VACCINE TRIALS
Kathleen Neuzil, University of Maryland, Baltimore, MD, USA
In 2020, the development of SARS-CoV-2 vaccines proceeded at a historic pace. Facing many challenges early on—a new disease poorly understood immunity, and an uncertain trajectory of the outbreak—a U.S. government effort brought together many partners to develop and advance several SARS-CoV2 vaccines. The vaccine development model included harmonized trial designs, collaborating clinical trial networks, and collaborating laboratory and statistical efforts. As of February 2021, two mRNA vaccines are being distributed under FDA Emergency Use Authorization (EUA) and several other vaccines are in various stages of clinical testing. Properly interpreting and comparing estimates of efficacy in these trials requires an understanding of the many variables involved and their potential effect on efficacy estimates. This talk will review the framework for the U.S. Covid-19 trials and discuss challenges and lessons learned.

75 EQUIitable ROLL-OUT OF COVID-19 VACCINES IN THE UNITED STATES
Michelle Williams, Harvard TH Chan School of Public Health, Boston, MA, USA
The United States is experiencing an intense reckoning with structural racism, in part because of the disparate health effects of COVID-19 on racial minorities suffering from higher rates of infection, hospitalization, and death. Yet despite equity efforts, we are already seeing racial disparities in vaccine distribution across the country. Going forward, vaccination efforts should prioritize economically worse-off racial minorities, not only to mitigate the disproportionate impacts of the pandemic, but also to help alleviate the country’s racial health disparities at large.

76 VACCINE ACCEPTANCE
Saad Omer, Yale University, New Haven, CT, USA
Vaccine acceptance is a complex phenomenon that lies on a continuum with those who actively seek vaccination on one end and those who refuse all vaccines on the other. When we talk about vaccine hesitancy, we mean those who accept some, delay some, or refuse some vaccines. Where one lies on this continuum is the result of an interaction between many different individual factors, such as values, cognitive biases, and trust, as well as environmental factors, like social norms, affordability, and convenience. There are different approaches to reducing vaccine hesitancy, some of which rely on taking advantage of cognitive biases, and others that use social norms, moral inclinations, and trust. This presentation will explore how we can use these different approaches to increase vaccine uptake.

77 GLOBAL DISTRIBUTION OF THE SARS-CoV-2 VACCINE
Nicole Lurie, Coalition for Epidemic Preparedness Innovations, Washington, DC, USA
This talk will describe the efforts of COVAX, a coalition of CEPI, Gavi and WHO, to develop, procure, and distribute SARS-CoV-2 vaccines for the world, with the goal of ensuring equitable access to those vaccines.
We found that nuclear capsids retained their integrity until shortly before integration occurs in the host nucleus. Nuclear RT generates functional viral DNA (vDNA), in fact, the ultimate goal of HIV-1 is integration into the host chromatin to optimize the release of high levels of viral progeny and discretely coexist with the host.

Methods: To uncover the HIV-1 DNA fate in the nuclear landscape we directly tracked the vDNA and the viral RNA (vRNA) by coupling HIV-1ANCHOR technology with RNA FISH or MCP-MS2 RNA-tagging bacterial system.

Results: Our computational imaging analysis revealed that proviral forms are early located in proximity of the nuclear periphery of mitotic and non-mitotic cells. We also observed that HIV-1 infection prompts clustering formation of the host factor CPSF6, restructuring nuclear membraneless organelles, enriched in both viral proteins and speckle factors. Interestingly, we observed that integrase proteins are retained in CPSF6 clusters, while the late retrotranscribed DNA was excluded from HIV-induced membraneless organelles (HIV-1 MLods), indicating that those structures are not proviral sites, but orchestrate viral events prior to the integration step. In particular, we show that HIV-1 MLods are sites of nuclear RT. We also observed that HIV-1 MLods are in the vicinity of pre-existing LEDGF clusters. Importantly, we identified that actively transcribing proviruses localize, outside HIV-1 MLods, in LEDGF-abundant regions, known to be active chromatin sites.

Conclusion: This study highlights single functional host-proviral complexes in their nuclear landscape, which is markedly structured by HIV-1 to favor viral replication.

HIV-1 CAPSID RETAINS ITS INTEGRITY UNTIL MINUTES BEFORE UNCOATING IN THE NUCLEUS

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Background: HIV-1 capsid core disassembly (uncoating) is a prerequisite for viral DNA integration into the host genome and a promising target for antiviral therapy. We recently developed a method to directly label capsid protein (CA) with green fluorescent protein (GFP-CA) in infectious viral complexes and reported that HIV-1 cores that retained >94% of their CA entered the nucleus and uncoated near their integration site ≤1.5 hours before integration. However, whether the nuclear capsids lost their integrity by rupturing or a small loss of CA before capsid disassembly was unclear.

Methods: We utilized a previously reported vector in which GFP is inserted in HIV-1 Gag (iGFP); proteolytic processing efficiently releases GFP, some of which remains trapped inside capsids and serves as a fluid phase content marker that is released when the capsids lose their integrity. We used live-cell imaging to track GFP and core-associated mRuby-tagged cleavage and polyadenylation specificity factor 6 (mRuby-CPSF6).

Results: We found that nuclear capsids retained their integrity until shortly before integration and lost their GFP content marker ~1-3 minutes before loss of capsid-associated mRuby-CPSF6. In contrast, when CA was tagged with GFP, loss of GFP and mRuby-CPSF6 occurred simultaneously. Thus, capsids retain their integrity until just minutes before uncoating.

Conclusion: Our results indicate that HIV-1 evolved to retain its capsid integrity and maintain a separation between macromolecules in the viral core and the nuclear environment until uncoating occurs just before integration. These observations imply that intact HIV-1 capsids are imported through nuclear pores, that reverse transcription occurs in an intact capsid, and that interactions between the preintegration complex and LEDGF/p75, and possibly other host factors that facilitate integration, must occur within a short time frame between uncoating and integration.

TRAFFICKING OF HIV-1 ENVELOPE TO THE EHD1/MICAL-L1 TUBULAR-SORTING ENDOSOME

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Background: HIV-1 Env is directed to the plasma membrane (PM) through the secretory pathway, and then is rapidly endocytosed to early endosomal compartments. We have previously described the trafficking of Env to the endosomal recycling compartment (ERC). In this study, we sought to further define the components of the ERC that are required for incorporation of Env into HIV-1 particles. Sorting tubules enriched in phosphatidylinositol (4,5) biphosphate (PIP2), Ep5 homology domain-containing protein 1 (EHD1) and molecule interacting with Casl-like protein 1 (MICAL-L1) have been described. We sought to define the role of MICAL-L1/EHD1 tubular sorting endosomes in HIV-1 Env incorporation and replication.

Methods: By employing pre-warmed paraformaldehyde and low concentrations of detergent, we were able to reproduce the work of others demonstrating long tubular EHD1- and MICAL-L1-positive compartments. We then performed colocalization analysis with markers of tubular endosomes and HIV-1Env. We used fluorogen-activated peptide (FAP) tagging to evaluate the kinetics of movement of Env to this compartment in living cells. We then used short hairpin RNA (shRNA) to deplete MICAL-L1 and EHD1 in HeLa cells and the H9 T cell line, and examined the incorporation of Env into particles and replication curves in wildtype vs. knockdown cells.

Results: Live cell TIRF microscopy revealed that pulse-labeled Env on the PM is rapidly segregated into PIP2-enriched tubules underlying the PM. In lightly-fixed cells, fluorescence colocalization analysis revealed a striking colocalization of HIV-1 Env with Rab10, EHD1, and MICAL-L1 in tubular sorting endosomes. Trafficking of Env lacking the cytoplasmic tail to tubular sorting endosomes, in contrast, was markedly diminished as compared with wildtype Env. Knockdown of EHD1 or MICAL-L1 in HeLa cells had little effect on Env incorporation. Strikingly, however, knockdown in H9 T cells resulted in production of particles with a deficiency of Env incorporation. Replication of HIV-1 was markedly diminished in EHD1- or MICAL-L1-depleted H9 cells as compared to control shRNA-treated cells.

Conclusion: We show here for the first time that Env colocalizes strongly with Rab10/EHD1/MICAL-L1-associated sorting tubules, and that Env particle incorporation and HIV-1 replication are dependent on EHD1 and MICAL-L1. We postulate that the tubular endosome compartment plays an important role in Env endocytosis and slow recycling back to specific sites on the PM for virus assembly.

A COMPREHENSIVE CRISPR SCREEN FOR HIV DEPENDENCY FACTORS

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Background: At each stage of the HIV life cycle, host cellular proteins are hijacked by the virus to establish and enhance infection. Although there have been many host factors identified to be important for supporting HIV infection (HIV dependency factors), there is still an incomplete understanding of all host factors involved.

Methods: To comprehensively identify genes that encode these dependency factors, the Emerman Lab has developed a virus-packagable HIV-CRISPR screen that utilizes a lentiviral vector containing a repaired long terminal repeat and is thus able to package into new virions. Packaging of these guideRNAs into HIV-1 particles serves as barcodes to identify which gene targets are required for successful HIV infection. Here, we have performed two independent HIV-CRISPR screens to comprehensively study cellular factors at the genome-wide level, using the Toronto Knockout version 3 (TKOv3) library, and targeted human epigenomic/epigenetic (HuEpi) library, in Jurkat T lymphocyte cells. We then curated the results of the top ~400 hits to make new libraries to be used to interrogate HIV dependency factors across multiple HIV strains.

Results: As positive controls, many known HIV dependency factors across multiple parts of the virus cycle were identified including the HIV receptor, co-receptor, LEDGF/p75, NFKB, and many genes encoding components of the Mediator complex. Notably, there were also numerous genes not previously reported to play a role in HIV biology involved in protein degradation, as well as epigenetic factors such as HDAC, KMT2A, and KMT2D. Therefore, this screening approach is sensitive enough to identify host factors for HIV involved at all parts
of the life-cycle and provides ample opportunity to investigate novel gene roles in HIV replication. Initial validation experiments confirm the involvement of top hits of the screen in HIV replication.

Conclusion: This iterative screening approach has repeatedly identified specific genes that may play novel biological roles during HIV infection. Using these data to inform creation of a focused library of presumed dependency factors, I am now conducting HIV-CRISPR screens and am validating important gene candidates, to create a comprehensive list of dependency factors common across cell types for multiple strains of HIV-1. This will both advance our understanding of the HIV-1 life cycle by identifying novel dependency factors required at different stages of the life cycle and has potential to inform therapeutic and cure design.

82 EARLY bNAb THERAPY IN SHIVAD8-EO-INFECTED Rhesus Macaques
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Background: Co-administration of the anti-HIV-1 bNAbs 10-1074 and 3BNC117 or treated at days 3, 10, and 17 post-challenge with either WT VRC07-523-LS and PGT121 (n=6) or their DEL mutants (n=6), which show increased binding to FcgRs in vitro.

Methods: SHIVAD8-EO intrarectally-challenged NHPs were not treated (n=6) or treated at days 3, 10, and 17 post-challenge with either WT VRC07-523-LS and PGT121 (n=6) or their DEL mutants (n=6), which showed increased binding to FcgRs in vitro.

Results: Five each of the 6 untreated or WT bNAb-treated monkeys became infected, but WT bNAb-treated monkeys suppressed virus for the first 8-14 weeks post-challenge. Viremia occurred after plasma bNAb levels declined. However, upon rebound, the peak and set point viral loads in these monkeys were indistinguishable from the untreated monkeys up to 124 weeks post-challenge. In contrast, only 3 monkeys treated with the DEL bNAbs rebounded so far. This occurred earlier than in the WT bNAb-treated monkeys (at 5-6 weeks post-challenge), associated with poor plasma pharmacokinetics of the DEL bNAbs. The other 3 DEL bNAb-treated monkeys have not rebounded 68 weeks post-challenge, despite absence of circulating bNAbs. Lymph node SIV-specific CD8+ T cell responses developed later in treated monkeys than in untreated animals, consistent with delayed plasma viremia upon bNAb therapy. RNA sequencing analysis of antigen-presenting cells from lymph nodes revealed that treated monkeys developed a specific transcriptional profile at week 2 post-challenge that persisted in DEL bNAb-treated monkeys through week 8 but was absent in untreated and WT bNAb-treated monkeys at the same timepoint.

Conclusion: No long-term differences in viral control were observed between untreated and WT bNAb-treated monkeys but 3 DEL bNAb-treated aviremic monkeys have not rebounded and are being studied more in depth to determine if they are controlling or completely free of virus. RNA sequencing analysis will be expanded to CD8+ T cell subsets to determine the effect of bNAb treatment in cell populations downstream of antigen-presenting cells, and microscopy will be conducted to determine the presence and location of the virus and infused bNAbs in the lymph nodes of these animals.

83 IMMUNE CORRELATES OF POSTTREATMENT CONTROL IN SHIV-INFECTED INFANT MACAQUES
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Background: Breastfeeding transmission accounts for the majority of new pediatric HIV infections and commits infants to lifelong ART, as interruption is typically followed by return of replication and repopulation of reservoirs. Previous studies in adult humans and animal models have identified correlates of viral control following analytical treatment interruption (ATI). However, it is critical to understand the kinetics and predictors of viral rebound in the setting of breastfeeding transmission to inform the development of novel pediatric-focused remission strategies.

Methods: At 4 wks of age, Mamu-A*01-/B*17+/A*01- or + rhesus macaques were orally administered SHIV.C.H05S.375H.dCT and placed on daily ART at 2 wpi (Intermediate, n=10) or 8 wpi (Late, n=10). ART was interrupted after 1 yr to assess viral rebound. Blood and lymph nodes (LN) were collected throughout the study for flow cytometry and viral measurements. Spearman correlations were used to identify associations between viral control and 30 immune parameters measured pre-ATI.

Results: Pre-ATI cell-associated SHIV DNA levels were 14-fold lower in blood (p=0.0030) and 60-fold lower in LN (p=0.0001) in animals that initiated ART earlier in infection, but this did not significantly delay time to viral rebound when compared to animals treated later. In both groups, rebound viremia was detected within 7-35 d post-ATI in all but 1 non-rebinder macaque. Post-treatment control (PTC) of viremia within 98 d of ATI was observed in 9/9 Mamu-A*01- macaques, an allele known to be associated with CTL responses to an immunodominant Gag epitope. However, PTC was also seen in 5/11 Mamu-A*01- macaques, suggesting viral control could be mediated by other mechanisms. We found that the frequency of Granzyme B+ KI67- NK cells (CD3- NKG2A+) in the blood pre-ATI was negatively correlated with area under the curve (AUC) for viral loads during rebound in Mamu-A*01- macaques (r=-0.8424, raw p=0.004). In addition, Mamu-A*01- animals with lower rebound viral load AUC tended to have a higher percentage of anti-Gag IFN-γ-producing CD4+ T cells in the blood (r=0.6126, raw p=0.05) and LN (r=-0.8424, raw p=0.01) prior to ATI.

Conclusion: This work provides novel insight into the predictors of PTC in a preclinical NHP model of pediatric HIV-1 infection. Our findings highlight aspects of innate and adaptive immunity may be critical in controlling viral rebound and should be studied further in the development of remission strategies in HIV-1-infected children.

84 SIVAG INFECTIVITY INDUCES ELITE CONTROL AND RESISTANCE TO SHIV HETEROLOGOUS CHALLENGE
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Background: HIV reservoir persistence is an obstacle to cure. Elite controllers (ECs) suppress virus in the absence of anti-retroviral drugs. Immunologic mechanisms that lead to viral control and interventions that could reduce or eliminate HIV reservoirs in ECs are unknown. Relevant models of EC in nonhuman primates (NHPs) could be critical to address these questions. Our lab has described and published a unique NHP model of elite SIV control termed ΔGY. This variant of SIVmac239 contains a 2 amino acid deletion in a preclinical NHP model of pediatric HIV-1 infection. Our findings highlight aspects of innate and adaptive immunity may be critical in controlling viral rebound and should be studied further in the development of remission strategies in HIV-1-infected children.
Methods: Pigtail macaques were infected with ΔGY (n=8) or SIVmac239 (n=4) and PBMC, lymph node (LN), duodenal tissues and plasma were collected longitudinally to identify transcriptional signatures and cytokines correlating with viral control, respectively (Fig1). Animals were challenged at week 80 with SHIV-SF162P3N.

Results: Gene signatures (all tissues) and cytokine/chemokine profiles at day 2 post-infection discriminated both groups while peak viral loads were similar. Compared to SIVmac239, ΔGY-infected animals presented decreased inflammasome signature/ cytokines (IL1b, IL1B), decreased anti-inflammatory signature/ cytokines (TGFb1, TGFb2) and increased homeostatic signaling downstream of IL2. Additionally, NLRX1, an early antagonist of type I IFN anti-viral signaling, was decreased in ΔGY animals and this was concomitant with higher CD8 effector function signatures (TBX21, IFNG, GZMB), lower T cell exhaustion (TOX, PDCD1) and preserved chemokine signaling (CXCL1, CXCL10-12). Correlates of these findings in ΔGY-infected animals included 1) control of viremia by 10-20 weeks; 2) normal numbers of CD4 T cells in blood and gut; and 3) lack of immune activation signatures. Additionally, when 6 ΔGY-controllers were challenged i.v. at 80 weeks with SHIV-SF162P3N, they controlled the challenge for more than 2 years (set points of ≤10^2-3 RNA cps/mL) in contrast to 4 naïve controls (set points 10^5-10^6 cps/mL).

Conclusion: ΔGY infection leads to a preserved adaptive immune response signature, in association with sustained viral control, CD4 T cell counts and resistance to a pathogenic i.v. heterologous challenge. Understanding of the mechanisms by which ΔGY infection can augment host immune responses to resist challenge viruses could have important implications for both HIV vaccine and pathogenesis fields.

85 TRAPPING THE HIV-1 V3 LOOP IN A HELICAL CONFORMATION ENABLES BROAD NEUTRALIZATION

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Background: Only a fraction of individuals develops broadly neutralizing antibodies (bnAbs) targeting the V3 base on a closed Env. In the CD4-bound conformation, the V3 becomes fully accessible and conformationally dynamic. Functional relevance of these intermediate V3 conformations and their potential for broad neutralization still remain to be resolved.

Methods: Here we applied the Designed Ankyrin Repeat Protein (DARPin) technology to select DARPin targeting HIV-1 Env by Ribosome Display. Hits were scored for V3 binders with broad neutralizing capacity. Neutralization breadth was assessed on a 42-multiclad Tier-2 virus panel in the T2M-bl assay. DARPin epitopes were characterized by binding to Env derivatives, deep mutational Env scanning, X-ray crystallography, cryo-EM and molecular dynamics.

Results: We identified 8 distinct V3 specific DARPin with exceptional neutralization breadth of up to 93%. Unlike V3-glycan bnAbs, these broadly neutralizing DARPin (bnDs) bound V3 solely on open but not closed Env. X-ray and cryo-EM structure analyses of bnD.8 and bnD.9 revealed binding to a 3-turn amphipathic alpha-helix in the C-Strand of V3 spanning residues 314 to 324. We termed this novel conformation αV3C. Remarkably, the αV3C helix was trapped with two unrelated bnDs and observed both in complex with V3 peptide and open, CD4-activated Env trimers. Molecular dynamics simulations indicated that the αV3C helix remains stable in the absence of the bnDs, emphasizing a functional relevance. Comprehensive Env mutation scanning underlined functional importance. Escape mutations accumulated on the contact face of the helix, but no enrichment of putative helix disturbing mutations occurred.

Conclusion: The discovery of post-CD4 engagement acting V3 inhibitors with extraordinary breadth is remarkable. The helical V3 conformation they define sheds light on V3 conformational dynamics after CD4 engagement and reveals a new site of vulnerability on HIV-1 Env. Our findings emphasize the importance of V3 and the open Env conformation as a target for inhibitors and mark the newly defined αV3C helix as a blueprint for epitope-based vaccine design.

AN Env-gag mRNA VACCINE PROTECTS MACAQUES FROM HETEROLOGOUS TIER-2 SHIV INFECTION

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Background: The development of a preventive vaccine remains a critical priority for ending the HIV/AIDS pandemic. Critical improvements in mRNA technology, as attested by recent successes in preventing COVID-19 disease, led us to develop an mRNA platform for HIV vaccines.

Methods: In this regard, we designed an mRNA vaccine with different HIV-1 envelope mRNAs from 3 different clades co-formulated with SIV gag mRNA, which can assemble virus like particles (VLPs) in vivo. Rhesus macaques were primed with a transmitted-founder clade-B Env lacking the 276 N-glycan followed by multiple glycan-repaired autologous and bivalent heterologous (clades A and C) booster immunizations.

Results: Immunized animals rapidly developed autologous neutralizing antibodies and eventually, after the second heterologous boost, cross-reactive tier-2 neutralizing antibodies, albeit at low titers. Vaccinated animals were protected from repeated low-dose rectal challenges with a heterologous tier-2 simian-human immunodeficiency virus (SIVΔ8). Protection was correlated with the presence of antibodies to the CD4-binding site.

Conclusion: Thus, the Gag-Env VLP mRNA platform offers a promising strategy for the development of an HIV vaccine.
ISLATRAVIR PK THRESHOLD & DOSE SELECTION FOR MONTHLY ORAL HIV-1 PrEP

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Background: Innovations in HIV-1 pre-exposure prophylaxis (PrEP) are needed to address the global HIV epidemic and meet the diverse needs of individuals at risk of acquiring HIV-1. Islatravir (ISL) is the first nucleoside reverse transcriptase translocation inhibitor (NRTTI) in development for the treatment and prevention of HIV-1. In this PK/PD work, we present the data that defined the exposure threshold for ISL for PrEP and the corresponding oral once monthly (QM) dose for the phase 3 clinical development program.

Methods: The lower efficacious exposure threshold for ISL PrEP efficacy was based on (1) the estimated protective ISL-triphosphate (ISL-TP) EC50, from a study of male rhesus macaque undergoing SHIV intra-vaginal challenge using once weekly oral ISL, (2) the minimum efficacious ISL-TP concentration in a Phase 1b proof-of-concept trial (NCT02217904) in treatment-naive adults with HIV-1, and (3) relevant data from the literature regarding the protective concentrations of FTC/TDF. Population PK simulations were conducted using data from the Phase 1 and 2 studies of ISL to determine the phase 3 oral QM dose.

Results: Totality of the data from the rhesus macaque study, the Phase 1b trial, and benchmarking PK exposures from TDF-DP resulting from both pre-clinical and clinical studies suggest that full efficacy for HIV-1 prevention is achieved with approximate inhibitory quotient (IQ) of ~1-5 (Table). Based on these observations, the efficacious exposure threshold for ISL PrEP was set at 0.05 pmol/106 cells in PBMCs, which is ~5 fold above the in vitro IC50 (0.000974 pmol/106 cells) of ISL-TP against wild type HIV-1. In an ongoing Phase 2 trial (NCT04003103), the observed mean ISL-TP exposure 4 weeks after a 60-mg dose was ~26 fold above the PK threshold. Additionally, population PK simulations suggest that oral ISL 60-mg QM will achieve ISL-TP concentrations well above the PK threshold for all participants following the first monthly dose. All participants at this dose are predicted to be above the PK threshold with the lower 2.5th prediction interval having an ~IQ of 17.

Conclusion: To provide efficacious exposures for protection against HIV-1, a 60-mg oral QM dose of ISL was selected for the Phase 3 clinical program. This dose is expected to maintain ISL-TP concentrations above the conservative PK threshold of 0.05 pmol/106 cells for all participants with sustained exposures in the event of a delayed or missed monthly dose.

CLINICAL EVALUATION OF DRUG INTERACTIONS WITH ORAL LENACAPAVIR AND PROBE DRUGS

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Background: Lenacapavir (LEN; GS-6207) is a potent, selective, first-in-class, multi-stage inhibitor of HIV-1 capsid function in clinical development as a long-acting agent for treatment and prevention of HIV-1 infection. Based on in vitro data, LEN is a substrate for P-gp, CYP3A, and UGT1A1, may inhibit BCRP, and may induce CYP3A, and may induce CYP3A. Also, LEN exhibits pH-dependent solubility (higher solubility at pH ≥6). In this Phase 1 study, we evaluated LEN drug interactions using cobicistat (COBI) +/- darunavir (DRV) as inhibitors of CYP3A/P-gp, voriconazole (VORI) as an inhibitor of CYP3A, rifampin (RIF) as an inducer of CYP/P-gp/UGT, and famotidine (FAM) as a gastric acid-reducing agent. The perpetrator interaction potential was evaluated using pitavastatin (PIT; OATP substrate), rosuvastatin (ROS; BCRP/OATP substrate), tenofovir alafenamide (TAF; P-gp substrate), and midazolam (MDZ; CYP3A substrate).

Methods: In separate cohorts of healthy participants (parallel study design), single oral doses of LEN (300mg) were given with and without COBI (150mg QD, Days 1-35), DRV/CBO (800mg/150mg QD, Days 1-35), VORI (400mg BID x 1 day, then 200mg BID Days 2-25), RIF (600mg QD, Days 1-25), and FAM (400mg single dose 2 h prior to LEN). In another cohort, single doses of PIT (2mg), ROS (5mg), TAF (25mg), and MDZ (2.5mg) were given prior to and simultaneous with LEN 600mg BID x 2 days, then Q3 days to rapidly achieve clinically relevant LEN exposure.

Results: Geometric-mean squares mean ratio (GLSM) and 90% confidence intervals (90% CI) for AUCmax (±20% study level) were: 1.048 (0.89-1.20) for LEN, 0.158 (0.13-0.19) for TAF, 0.428 (0.28-0.65) for MDZ, and 0.892 (0.75-1.05) for PIT. There were no differences in maximum tolerated dose of Lenacapavir with or without probe drugs.
91 PK OF DOSE-ADJUSTED EMERGENCY CONTRACEPTION WITH EFV-BASED ART IN ACTG 5375

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Background: Levonorgestrel (LNG) emergency contraception (EC), when administered as a single dose, prevents pregnancy by delaying ovulation after unprotected sex that could lead to pregnancy. LNG area under the concentration time curve (AUC) is reduced 57% when coadministered with EFV in healthy volunteers. Some guidelines recommend dose-adjustment of LNG EC from 1.5mg to 3mg when combined with EFV, but this strategy has not been assessed in pharmacokinetic (PK) or clinical studies. We hypothesized that doubling the dose of LNG EC to 3mg would increase LNG exposure in individuals receiving EFV-based ART.

Methods: ACTG study A5375 was a multicenter, parallel group, PK evaluation of pre-menopausal females, ≥16 years old, without an indication for EC at entry. Participants with HIV and receiving EFV plus two NRTIs were randomized (1:2 ratio) to either LNG 1.5mg (n=17) or 3mg (n=35), given as a single dose with food. Plasma was collected pre-dose, then 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, and 48h post-LNG dose. Participants were followed for 4 weeks to assess adverse events. LNG concentrations were measured by LC-MS/MS and PK parameters calculated by non-compartmental methods. PK parameters were compared between dosing groups by geometric mean ratio (GMR; 90% CI).

Results: Participants (n=52) enrolled between Sept and Dec 2019. All self-identified as cis-women, 29 (56%) Asian, 19 (37%) African, and 4 (8%) Latina, with mean (SD) BMI 23.9 (5.6) kg/m². The Table summarizes LNG PK parameters. The maximum concentration (Cmax) was 51% higher after LNG 3mg (24.9 mg/mL) compared to 1.5mg (15.1 mg/mL), and the 48h concentration was 133% higher (0.6 vs 0.3 mg/mL, respectively). The AUC over 8 hours was 66% higher in the 3mg group and remained 74-77% higher over 24 and 48 hours post-dose. Other PK parameters did not differ between groups (Table). No targeted or severe adverse events were reported.

Conclusion: EFV induced LNG metabolism, as demonstrated by a 12h half-life in both study groups, compared to 27h in historic data in the absence of a drug-drug interaction (Praditpan et al, Contraception 2017). Dose adjustment of LNG EC from 1.5mg to 3mg successfully increased LNG exposure in women receiving EFV-based ART. In combination with EFV-based ART, LNG 3mg resulted in a higher Cmax both herein and compared to historic data of LNG 1.5mg (18.2 mg/mL; Praditpan et al). As Cmax is the proposed PK parameter associated with EC effectiveness, our data support the dose-adjustment of LNG EC to 3mg in women receiving EFV-based ART.
EMTRICITABINE TRIPHOSPHATE IN DRIED BLOOD SPOTS PREDICTS FUTURE VIREMIA IN PWH

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Background: The quantification of emtricitabine triphosphate (FTC-TP) in dried blood spots (DBS) is a recent adherence measure due to its 35-hour half-life in this matrix. In persons living with HIV (PWH), it is associated with viral suppression and is predictive of 3-day adherence. However, its value to predict future viremia has not been evaluated.

Methods: DBS, HIV viral load (VL) and self-reported (SR) adherence were prospectively obtained in a clinical cohort of PWH receiving tenofovir disoproxil fumarate (TDF)-FTC-based antiretroviral therapy (ART), using convenience sampling at regular clinic visits (up to 3 visits over 48-weeks). FTC-TP concentrations in DBS were dichotomized into quantifiable vs. below the limit of quantification (BLQ). Generalized linear logistic regression was used to estimate the odds ratio (OR) of future viremia (>20 copies/mL) based on FTC-TP concentrations at the current visit. Additional models included adjustment for tenofovir diphosphate (TFV-DP) in DBS, given its known utility as a marker of adherence and its independent association with viral suppression and VL. We also compared fluctuations in TFV-DP levels in cases (prior to elevated VL) and controls.

Results: A total of 433 PWH (69 female, 83 Black, 85 Hispanic) contributed 677 paired DBS and HIV VL samples. The OR (95% CI) for future viremia for BLQ vs. quantifiable FTC-TP in DBS was 4.1 (2.3, 7.2; p<0.001, Table). This remained significant after adjusting for age, gender, race, body mass index, ART class, eGFR, hematocrit and CD4+ T cell count, aOR 3.4 (1.8, 6.5; p<0.0002, Table). In contrast, this association was not observed in PWH who reported <100% 3-day adherence, aOR 1.3 (0.4, 4.6; p=0.96, Table).

Conclusion: FTC-TP in DBS is a predictor of future viremia in PWH. This relationship, although diminished by including TFV-DP in the model, demonstrates the ability of a short-term adherence measure to predict future viremia. The mismatch in FTC-TP in DBS reporting 100% 3-day adherence suggests PWH who report high recent adherence, but who have an undetectable FTC-TP, could benefit from ART adherence counseling regarding their risk of future viremia.

Table. Odds ratio (OR) and adjusted OR (aOR) of risk of future HIV viral load >20 copies/mL, by concentration of emtricitabine triphosphate in dried blood spots at current visit in the study population (N=677), and after further adjusting for 3-day self-reported adherence (95% CI vs. 100%).

92 TENOFOVIR DIPHOSPHATE TO PREDICT FUTURE VIRAEMIA IN POSTPARTUM WOMEN LIVING WITH HIV

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Background: There are few data on how tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) predicts future viral load (VL) in people living with HIV on antiretroviral therapy (ART).

Methods: We conducted a nested case-control study within a trial of differentiated care for postpartum care in ART (NCT03200054). Women were >18 years, started ART (TDF+3TC+EFV) in pregnancy and were enrolled <10w postpartum with a recent VL<400 copies/mL. Samples for VL and TFV-DP assays were taken 3-6 months after enrolment. Cases were women with >1 VL<20 copies/mL and controls were a random sample of women with persistent viral suppression (VS; VL<20 copies/mL). We analysed how absolute TFV-DP levels during VS predicted subsequent risk of VS; results are expressed as sensitivity (SE), specificity (SP) and test likelihood odds ratios (LORs). We also compared fluctuations in TFV-DP levels in cases (prior to elevated VL) and controls.

Results: Overall 81 women and 365 visits were included (median age: 29y, median time on ART: 6m; median time postpartum: 19d). The median duration between each TFV-DP and VL measure was 175d (IQR: 92-184). TFV-DP levels measured during VS were associated with risk of viremia 3-6m later in a dose-response manner: 17% of women with TFV-DP >1850 fmol/punch had a VL>20 copies/mL at the next visit, compared to 23%, 25%, 48% and 91% of women with TFV-DP levels 1250-1849, 700-1249, 350-699 and <350 fmol/punch, respectively (Figure). Variance in TFV-DP levels during VS was significantly higher in cases vs controls (p<0.05). SE of TFV-DP>350 to predict VS at the next visit was 96% (95% CI: 93-98) and did not vary by time between measures or preceding VL values. SP of TFV-DP>350 to predict VS at the next visit was 54% (95% CI: 46-62) and was lower if the DBS was done <120 days before the next VL (36%, 95% CI 21-53). Adjusting for age, ART duration and time between TFV-DP and next VL measure, the ORs of VL>20 copies/mL for TFV-DP levels 1250-1849, 700-1249, 350-699 and <350 fmol/punch compared to TFV-DP>1850 fmol/punch were 1.3 (95% CI: 0.6-3.2), 1.6 (95% CI: 0.7-3.3), 3.6 (95% CI: 1.3-10.0) and 28.8 (95% CI: 9.7-86.5), respectively; the associations persisted using alternate outcome definitions (VL>400 and >1000 copies/mL).

Conclusion: In postpartum women, TFV-DP levels during VS predict viremia 3-6m later, indicating the potential value of objective adherence markers in high-risk populations.
NUCLEOSIDES AND DARUNAVIR/DOLUTEGRAVIR IN AFRICA (NADIA) TRIAL: 48 WKS PRIMARY OUTCOME

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Background: WHO recommends dolutegravir with two NRTIs for second-line treatment of HIV infection after failure on an NNRTI-based regimen. There is limited evidence for efficacy of this dolutegravir regimen when prescribed NRTIs lack predicted activity due to drug resistance; or for the recommendation to switch from tenofovir to dolutegravir in second-line.

Methods: In a two-by-two factorial, open-label, non-inferiority trial, we randomized patients failing an NNRTI/tenofovir/lamivudine first-line regimen with confirmed VL ≥1000 copies/ml to receive dolutegravir or ritonavir-boosted darunavir; and to receive tenofovir or dolutegravir; all with lamivudine. Treatment was monitored by VL at 24 and 48 weeks, following WHO guidelines. Baseline NRTI resistance testing was batched, and results blinded. The primary outcome was the percentage of patients with week-48 VL <400 copies/ml using FDA snapshot algorithm (non-inferiority margin 12%).

Results: We enrolled 464 patients at 7 sub-Saharan African sites (61% female, 51% C4<200, 28% VL≥100,000). At baseline, 58.5% overall had intermediate-high level resistance to tenofovir and 92% had resistance to lamivudine. Week 48 VL was <400 copies/ml in 92.2% in the dolutegravir group and 91.7% in the darunavir group (difference -0.5%, 95% CI: -1.2 to 0.2; P=0.497; indicating non-inferiority of dolutegravir, without superiority). In the subgroup with no predicted-active NRTIs in the prescribed regimen, VL was <400 copies/ml in 92.4% of those in the dolutegravir group and 93.7% of those in the darunavir group. To date, 3 have intermediate-high level darunavir resistance; 0 have darunavir resistance. In the other randomized comparison, VL was <400 copies/ml in 92.3% in the tenofovir group and 89.6% in the dolutegravir group (difference 2.7%, 95% CI: -2.6 to 7.9%; P=0.327).

Conclusion: Dolutegravir with two NRTIs gives highly effective viral suppression to 48 weeks, even in a patient population where many have extensive NRTI resistance and no predicted activity in prescribed NRTIs. This finding is important for patients switching from NNRTI to dolutegravir with NRTIs after known treatment failure; and for programmes switching stable patients systematically from NNRTI to dolutegravir-based regimens without VL and resistance testing. Tenofovir can be maintained in second-line therapy without switching to dolutegravir, with advantages for patients and programmes.

95 RANDOMIZED TRIAL OF RESISTANCE TESTING FOR VIROLOGIC FAILURE IN SUB-SAHARAN AFRICA

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Background: Genotypic resistance testing (GRT) is recommended after virologic failure in resource-rich settings. By contrast, guidelines in sub-Saharan Africa promote adherence support and repeat virologic monitoring, in the absence of GRT, to guide treatment.

Methods: We conducted an open-label, randomized controlled trial to assess whether GRT improved virologic suppression after first-line antiretroviral therapy (ART) failure in sub-Saharan Africa. We enrolled adults in ambulatory care in South Africa and Uganda on first-line ART with an HIV-1 RNA viral load (VL) >1,000 copies/ml. We excluded those currently eligible for second-line or with prior known drug resistance. Individuals were randomized 1:1 to standard-of-care (SOC), with adherence counseling and virologic monitoring to determine management, or immediate GRT, with therapeutic decision making by clinical staff trained in GRT interpretation. The primary outcome was achievement of VL<200 copies/ml 9 months after enrollment. Analyses were intent-to-treat such that those deceased or lost from care were considered failures. Secondary outcomes included suppression less than assay, suppression while maintaining first-line ART, retention in care, mortality, and presence of drug resistance at study conclusion among those with a VL>1,000 copies/ml.

Results: We enrolled 840 participants, divided equally by country and sex. The median age was 37 years, median ART duration was 3 years, and 82% were taking efavirenz-based ART. There was no difference in the proportion in care and achieving a VL<200 copies/ml at 9 months by arm (SOC: 256/423, 61%; RT: 263/417, 63%, OR 1.11, 95% CI 0.83-1.49, P=0.46). Results were similar in pre-defined sub-groups (Figure). Those with a VL<1,000 copies/ml at 9 months in the SOC arm were more likely to have drug resistance detected (SOC: 75/103, 73%, OR 1.15, 95% CI 0.90-1.46, P=0.23; 33/46, 72%, OR 1.11, 95% CI 0.83-1.49, P=0.46). Results were similar in pre-defined sub-groups (Figure). Those with a VL<1,000 copies/ml at 9 months in the SOC arm were more likely to have drug resistance detected (SOC: 75/103, 73%, OR 1.15, 95% CI 0.90-1.46, P=0.23; 33/46, 72%, OR 1.11, 95% CI 0.83-1.49, P=0.46). Results were similar in pre-defined sub-groups (Figure). Those with a VL<1,000 copies/ml at 9 months in the SOC arm were more likely to have drug resistance detected (SOC: 75/103, 73%, OR 1.15, 95% CI 0.90-1.46, P=0.23; 33/46, 72%, OR 1.11, 95% CI 0.83-1.49, P=0.46). Results were similar in pre-defined sub-groups (Figure). Those with a VL<1,000 copies/ml at 9 months in the SOC arm were more likely to have drug resistance detected (SOC: 75/103, 73%, OR 1.15, 95% CI 0.90-1.46, P=0.23; 33/46, 72%, OR 1.11, 95% CI 0.83-1.49, P=0.46).

Conclusion: In public clinics in sub-Saharan Africa, the addition of GRT to routine care did not improve achievement of virologic suppression 9 months after first-line ART failure but did lower the likelihood of drug resistance in those with persistent viremia. Interventions that improve management of ART failure remain elusive and are of particular importance to enable prompt transition from efavirenz-based to dolutegravir-based therapy in the region.
SHORT-COURSE ALENDRONATE FOR THE PREVENTION OF ART-ASSOCIATED BONE LOSS

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Background: Antiretroviral therapy (ART) initiation encompasses a period of accelerated BMD loss in people with HIV (PWH). The Alendronate for the Prevention of ART associated bone loss (APART) study aimed to evaluate if short-term use of the oral, generic bisphosphonate alendronate (ALN) could prevent BMD loss at ART initiation.

Methods: In this multisite, double blinded, placebo-controlled phase 4 clinical trial, ART-naive, PWH initiating ART with tenofovir disoproxil fumarate/entecitabine and emtricitabine and a third agent were randomized to calcium/vitamin D3 supplementation with either generic, oral ALN 70mg weekly or placebo (PL), for 2 weeks prior to ART initiation and for a total of 14 weeks. Clinical, laboratory, safety and BMD at lumbar spine (LS) and total hip (TH) were assessed at weeks 0, 14, 26 and 50. Primary endpoint was between group % change in BMD from baseline (BL) to week 50, compared using Wilcoxon rank tests. Secondary endpoints included % change from BL at weeks 14 and 26.

Results: Of 50 subjects randomized (ALN N=24, PL N=26) 86% were male, 46% Caucasian, 34% African and 20% Hispanic, median age was 35 (32, 40) years and BMI 24 (22.3, 26.9) kg/m². Third agent use comprised integrase inhibitors in 94% and protease inhibitors in 4%. BL BMD was not significantly different between groups. At week 50, subjects in the ALN group had a median 0.5% (-3.10, 1.80) increase in TH BMD compared to a 2.7% (-4.3, -0.20) decrease in the PL group (between group difference P=0.02, Fig 1a). At the LS, the ALN group had a 1.4% (-4.10, 3.13) loss compared to 3.69% (-4.82, -1.7) loss in the PL group (between group difference P=0.02, Fig 1a). TH BMD between-group differences were evident early at week 14 (+1.88% (-0.7, 2.81) with ALN vs -0.65% (-2.65, 1.13) with PL, P=0.036) and persisted to week 50 (Fig 1a). In contrast, at the LS, between group differences although evident at weeks 14 (+1.24% (-0.4, 3.02) with ALN vs -0.96% (-3.10, 0.78) with PL, P=0.013) and 26 (ALN +0.05% (-3.04, 3.3) vs PL -2.48% (-4.65, -0.24), P=0.03) these didn’t persist to week 50 (Fig 1b). ALN was well tolerated with no significant differences in adverse events between the groups.

Conclusion: In this multisite, clinical trial, 14 weeks of oral alendronate, commenced prior to ART initiation, had a sustained impact on prevention of ART-associated bone loss at the TH, while the protective effect at the LS was limited to the first 24 weeks. These data support the use of short-course, generic alendronate to preserve BMD in PWH initiating ART.

PREVENTION OF CARDIOVASCULAR DISEASE IN PERSONS WITH AND WITHOUT HIV

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Background: With higher risk of cardiovascular disease (CVD) in persons with HIV (PWH), management of hypertension, dyslipidemia, and diabetes mellitus is crucial. Here, we evaluate the extent to which PWH have successfully managed these conditions and their influence on CVD risk.

Methods: Cohort study of adult (≥ 18 years) PWH and 20:1 age-, sex-, race/ethnicity-matched persons without HIV (PWoH) who were members of an integrated healthcare system in Northern California during 2013-2017. We excluded subjects with prevalent CVD (coronary heart disease or ischemic stroke). In those with hypertension, dyslipidemia, and diabetes, we computed the disease management index (DMI), which accounts for both the amount and duration of person-time above treatment goal (vertical lines in Figure) over 6-month intervals. A DMI of 100% represents perfect control and DMI <100% is the amount in control relative to a reference treated population. Next, using Cox regression, we computed hazard ratios (HR) for incident CVD by HIV status overall, and in subgroups of those with successfully controlled risk factors (i.e., DMI 100%). Models were adjusted for other key modifiable risk factors (smoking and alcohol use), demographics, and clinical factors (Charlson comorbidity index, depression, body mass index, healthcare utilization).

Results: The study included 8,285 PWH and 170,517 PWoH, with similar prevalences of hypertension (19% PWH; 22% PWoH), dyslipidemia (41% for both) and diabetes (8% PWH; 9% PWoH). PWH and PWoH had similar control of most conditions (with DMIs approaching 100%) except for triglycerides (worse control for PWH) and HbA1c (better control for PWH) (Figure). Among PWH, other factors, including smoking and unhealthy alcohol use, had only marginal associations with reduced DMIs. Overall, PWH had 450 CVD events (20.8 per 1,000 person-years) and PWoH had 7,648 events (170 per 1,000 person-years), with an adjusted HR of 1.18 (95% CI 1.07-1.30). The elevated risk of CVD for PWH...
was attenuated and not statistically significant when comparing PWH and PWoH with successfully controlled dyslipidemia (HR 1.10; 95% CI 0.91-1.34), and diabetes (HR 1.02; 0.72-1.42), but remained significant for those with successfully controlled hypertension (HR 1.35; 1.10-1.67).

Conclusion: Successful management of dyslipidemia and diabetes may help mitigate the CVD disparity in PWH. Further research is needed to evaluate whether more stringent hypertension treatment goals than <140/90 mm Hg are needed to prevent CVD in PWH.

Figure 1. Distribution of blood pressure, lipid, and HbA1c lab measurements

SEX MODIFIES THE ASSOCIATION BETWEEN INFLAMMATION AND VASCULAR EVENTS IN TREATED HIV


Background: Inflammation persists in people with HIV (PWH) despite antiretroviral therapy (ART) and predicts morbidity and mortality. The specific inflammatory pathways associated with myocardial infarction (MI), ischemic stroke, and venous thromboembolism (VTE), and whether these pathways are modified by sex, have yet to be fully described.

Methods: Using a case-cohort design, we randomly sampled all CNICS participants with available plasma after 1 year of ART-mediated viral suppression (the cohort), and from the same timepoint, all participants who were subsequently diagnosed with an incident type 1 or 2 MI (T1MI, T2MI), ischemic stroke, or VTE (all centrally adjudicated). Composite cardiovascular disease (CVD) event included T1MI or ischemic stroke. The relationship between 11 plasma biomarkers normalized to the cohort interquartile range (IQR) and subsequent event risk was assessed by logistic regression, adjusting for age, natal sex, nadir CD4, and other potential confounders (smoking, IDU, ASCVD risk score, and HCV history).

Results: We sampled a random sub-cohort of 979 (of 9430 eligible) participants and 103 CVD (75 T1MI, 30 ischemic stroke), 56 T2MI, and 80 VTE cases. In the sub-cohort, median age was 47, 82% were men, and 17% had a history of IDU. Median ASCVD risk was 4% and median current and nadir CD4 were 576 and 246, respectively. After adjustment, women had a 1.4-2.5 fold IQR higher CRP, LBP, sCD14, suPAR, ICAM-1, and CMV IgG than men (P<0.03 for all). Higher CRP, sCD14, and sTNFR2 were associated with subsequent T1MI (1.3-1.6 fold higher odds per IQR increase, P<0.01 for all), while higher CRP, suPAR, and ICAM-1 were associated with VTE (1.5-1.7 fold higher odds per IQR increase, P<0.01 for all). All biomarkers except CRP, LBP, and CMV IgG were associated with incident T2MI (1.4-2.8 fold greater odds per IQR increase, P<0.001 for all). Inflammatory markers tended to be associated with CVD events more strongly among women compared to men (KT ratio and sCD14 P for interaction = 0.005 and 0.08) but tended to be associated with VTE events more strongly in men than women (sCD14 and sTNFR2 P for interaction = 0.02 and 0.10).

Conclusion: Discrete pathways of inflammation independently predict CVD and VTE events in treated HIV, and many of these pathways are modified by sex. As inflammation predicts CVD events more strongly in women than in men, representation of women in clinical studies of immune-based interventions in treated HIV infection is critical.
100 BIOLOGICAL PROFILES PREDICT CORONARY ARTERY DISEASE IN PWH AND RISK-MATCHED CONTROLS

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Background: Inflammation has been implicated in the increased risk of coronary artery disease (CAD) observed in individuals with HIV (PWH) with the immune or inflammatory pathways contributing to CAD not well defined.

Methods: The HIV UPBEAT CAD sub-study enrolled PWH >40 years old on effective antiretroviral therapy (ART) and uninfected controls propensity matched for CAD risk. We used coronary computed tomography angiography (CCTA) to estimate subclinical CAD and performed chemiluminescence immunoassays to evaluate 28 biomarkers of systemic, innate and vascular inflammation and 10 T-cell immune markers by flow cytometry. Principal component (PC) analysis was used to reduce data dimensionality followed by PC-based unsupervised hierarchical clustering to partition participants into biomarker-derived clusters. Associations between clusters and subclinical CAD were explored using logistic regression with additional adjustment for HIV status. Data are median (interquartile range) unless specified.

Results: Of 101 participants (51% PWH), 72% were male, 75% Caucasian and median age 49 (45, 55) years. 35.6% had subclinical CAD (32% of PWH). We identified three clusters (figure 1a): Cluster 1 (n=41, 32% PWH) characterised by lower T-cell senescence and activation and lower TNF, TNF R1/2 and IL1ra; Cluster 2 (n=40, 72% PWH) characterised by higher T-cell senescence and exhaustion (higher T-cell senescence, activation, effector T cells, higher TNF R1/2, MIP, but lower IL2, IL12 and IFN); and Cluster 3 (n=19, 52% PWH) characterised by higher inflammation (higher IL2, IL12, IFN, IL4, IL10, TNF, IL6, IL1b, IL1ra, IFABP). With the exception of HIV status, baseline demographics were similar between clusters including CD4+ T-cell count and CD4+CD8+ ratio for PWH. Compared to those in Cluster 1, those in Cluster 2 and 3 had greater presence of any coronary plaque, partially calcified and calcified plaque, an association which for cluster 2 strengthened after adjustment for HIV status (figure 1b). In contrast, participants in Cluster 3 had higher calcification scores [Agatson score > 100, Calcium volume score > 100] which persisted in adjusted analyses.

Conclusion: In a cohort of CAD risk-matched individuals with and without HIV, we identified two clusters associated with distinct characteristics of subclinical CAD, one characterised by T cell senescence and exhaustion, the other by systemic inflammation. Biological phenotyping may help better predict those at risk of long term comorbidities common in ART treated PWH.

Table: Top Ten Prognostic Proteins for Mortality Among HIV-infected individuals in AIVACS

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>HR</th>
<th>p-value</th>
<th>q-value</th>
<th>Protein Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDF11</td>
<td>1.25</td>
<td>5.16E-36</td>
<td>8.37E-33</td>
<td>Bone morphogenetic protein 11</td>
</tr>
<tr>
<td>VMP1</td>
<td>1.29</td>
<td>6.22E-36</td>
<td>8.31E-33</td>
<td>Vascular permeability factor</td>
</tr>
<tr>
<td>ADAMTSL2</td>
<td>1.34</td>
<td>1.56E-32</td>
<td>1.18E-30</td>
<td>ADAMTS-like protein 2</td>
</tr>
<tr>
<td>VSEP1</td>
<td>1.37</td>
<td>1.70E-32</td>
<td>1.14E-29</td>
<td>Vascular permeability factor</td>
</tr>
<tr>
<td>SPOC2</td>
<td>1.37</td>
<td>5.05E-32</td>
<td>1.08E-29</td>
<td>Spodin-2</td>
</tr>
<tr>
<td>FHL1P1</td>
<td>1.37</td>
<td>7.80E-12</td>
<td>1.65E-09</td>
<td>EGF-containing fibulin-like matrix protein 1</td>
</tr>
<tr>
<td>EGFR</td>
<td>0.50</td>
<td>2.97E-11</td>
<td>7.64E-09</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>TSL1</td>
<td>1.37</td>
<td>1.32E-30</td>
<td>4.94E-28</td>
<td>Adhesion von Willebrand factor</td>
</tr>
<tr>
<td>ADAMTSL1</td>
<td>1.45</td>
<td>3.66E-20</td>
<td>1.08E-16</td>
<td>ADAMTS-like protein 1</td>
</tr>
<tr>
<td>SET</td>
<td>0.51</td>
<td>5.43E-15</td>
<td>1.44E-12</td>
<td>SET protein</td>
</tr>
</tbody>
</table>

Figure 1: (a) Characterization of biomarker derived clusters and (b) relationship with subclinical Cardiovascular disease

101 12-YEAR COGNITIVE DECLINE IS ASSOCIATED WITH LUNG DISEASE, DIABETES, AND DEPRESSION

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Background: Cognitive impairment is more common in people with HIV (PWH) than in the general population and is associated with worse quality of life and worse health outcomes. Most studies of cognitive change in PWH have focused on decline over a few years but no projects have assessed cognitive change and its correlates over more than a decade. To address this key gap, the 6-site,
At the 12-year visit, mean age was 56 (range 33-81), 23% were women, 58% had race/ethnicity other than white, and 96% took antiretroviral therapy (ART, mean 15.3 years) with mean CD4+ T-cell count 607/µL and plasma HIV RNA ≤ 200 cp/mL in 90%. The criterion for cognitive decline was met in 23.4%. In the best model, worse RBCS was associated with chronic lung disease (p=0.002) and lifetime cannabis use disorder (p=0.037) (model p<0.0001). The model also included an interaction between diabetes and major depressive disorder (MDD) (p=0.026); people with diabetes and MDD had worse cognitive decline than people with diabetes without MDD or people with neither condition. Hypertension also entered some models and a comorbidity index that combined it with diabetes and chronic lung disease was incrementally associated with decline (p<0.0001).

Conclusion: Nearly a quarter of treated PWH experienced cognitive decline over 12 years and worse change was associated with previously reported aging-related risk factors (e.g., diabetes) but also with other risk factors that have not been reported (chronic lung disease, MDD, cannabis use disorder). The CHARTER cohort was intended to reflect PWH who receive outpatient healthcare in the U.S. when it was first recruited but these 12-year findings may be affected by survivor bias and selection bias.

Results: Accompanying an increase in median age of PWH (50 to 53 years), the projected prevalence of multimorbidity increased from 2020 to 2030 overall (from 30.3% to 34.7%) and among all 15 key-populations (Figure). Racial disparities expand over time, with the highest and lowest multimorbidity burden projected among Black IDU women (74.7%) and Hispanic MSM (20.9%) in 2030. Hispanic heterosexual women experienced the largest multimorbidity burden increase (absolute difference=23.5%), while multimorbidity burden reduced slightly among White and Black heterosexual men (<2% change). Anxiety (mean prevalence [range]: 0.54 [0.12–0.82]) and depression (0.49 [0.26–0.64]) were among the most prevalent comorbidities across all key-populations in 2030, followed by hypertension (0.38 [0.15–0.59]), CKD (0.36 [0.13–0.88]) and diabetes (0.31 [0.11–0.48]).

Conclusion: The prevalence of multimorbidity in PWH is projected to increase over the next decade. The most prevalent comorbidities, particularly anxiety, depression, hypertension, CKD, and diabetes, differ by race/ethnicity, sex, and transmission category. Focusing on the most burdensome comorbidities in each key-population can help with the long-term care and life-expectancy of PWH.
viremia, it might also dampen the severe immune response to the virus; data comparing ISC groups is limited.

**Methods:** Using patient-level data from 34 sites in the U.S. National COVID Cohort Collaborative (N3C), we compared risk of COVID-19 hospitalization amongst COVID-19 patients in 3 ISC groups (1,300 persons with HIV [PWH]; 2,142 solid organ transplant [SOT] patients; 41 PWH with SOT) to 288,743 COVID-19 patients without HIV or SOT [HIV-/SOT-]. COVID-19+ was defined by RT-PCR, antibody test, or diagnostic codes. HIV infection, SOT and comorbidities were defined by conditions/diagnostic codes within 2 years prior to first COVID-19+. Hospitalization was defined by inpatient care between 14 days prior to 45 days after the first COVID-19+. Odds ratios of hospitalization were estimated using multivariable logistic regression models adjusting for demographics, study site, and comorbidities (severe liver disease, diabetes, cancer, kidney disease, and total comorbidities [0, 1, 2, ≥3]).

**Results:** Of 292,226 COVID-19+ patients, the median age was 41 years (IQR: 25-58), 46% male, 47% non-Hispanic white (NHW), and 17% non-Hispanic black (NHB). PWH and SOT patients, respectively, were more likely to be older (median: 50 & 56), male (70% & 60%), and had ≥3 comorbidities than overall N3C patients (30% & 64% vs. 8%). PWH were more likely to be NHB (50%) and SOT patients were more likely to be NHW (44%). Overall, 26% of HIV-/ SOT- COVID-19 patients were hospitalized. In crude analyses with HIV-/SOT- as the referent group (Table), COVID-19 patients with HIV, SOT or both had a 2.3, 4.4, or 6.9-fold increased odds of hospitalization, respectively. After adjustment for demographics and site, the risk was attenuated but remained statistically significant (Model a). Sequential adjustment for the type and number of comorbidities obviated the estimated risk among PWH, while SOT patients had persistently increased odds of hospitalization (Model b).

**Conclusion:** ISC patients (PWH and SOT) are more likely to be hospitalized with COVID-19 independent of demographics. However, this increased hospitalization risk was driven mainly by the high burden of comorbidities in both groups. Only SOT patients had an independent risk of hospitalization after adjusting for comorbidities. Ongoing analyses will examine the impact of ISC on additional COVID-19 outcomes (i.e. ventilation use, death).

### Table. Odds of hospitalization with COVID-19 for people with HIV, solid organ transplant or both compared to N3C patients in crude and adjusted models.

<table>
<thead>
<tr>
<th>Immune suppression groups</th>
<th>Crude estimates</th>
<th>Adjusted estimates a</th>
<th>Adjusted estimates b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWH: (N=1,305)</td>
<td>2.60 (2.09-3.26)</td>
<td>&lt;0.01</td>
<td>1.41 (1.24, 1.58)</td>
</tr>
<tr>
<td>SOT: (N=1,142)</td>
<td>3.10 (2.20-4.68)</td>
<td>&lt;0.01</td>
<td>1.50 (1.04-2.15)</td>
</tr>
<tr>
<td>HIV &amp; SOT+ (N=441)</td>
<td>1.97 (1.50-2.60)</td>
<td>&lt;0.01</td>
<td>1.04 (0.78-1.39)</td>
</tr>
</tbody>
</table>

*Model adjusted for age, sex, race and ethnicity (Black non-Hispanic, white Hispanic, white non-Hispanic, others), and study site.

1. **DISPARITIES IN TIMELY RECEIPT OF ART PRESCRIPTION IN HIV CARE IN THE US, 2012-2018**

**Jun Li**, Elizabeth Humes, David B. Hanna, Jennifer S. Lee, Ken N. Althoff, Richard Moore, Heidi Crane, Jonathan Golanski, Michael A. Horberg, Ank Nijhawan, Gypysam D’Souza, Christopher T. Rentsch, Kelly Gebo, Kate Buchacz, for the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA

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2. The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, Albert Einstein College of Medicine, Bronx, NY, USA
3. University of Washington, Seattle, WA, USA, Emory Center for AIDS Research, Atlanta, GA, USA, Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, University of Texas Southwestern, Dallas, TX, USA, VA Connecticut Healthcare System, West Haven, CT, USA

**Background:** Since 2012, the U.S. Department of Health and Human Services (DHHS) has recommended ART for all people with HIV (PWH) regardless of CD4 count. We studied trends in and sociodemographic and clinical disparities in timely receipt of ART prescription (ART) from 2012–2018.

**Methods:** We examined HIV treatment-naïve adults who newly presented to HIV care (i.e., HIV viral load [VL] >50 copies/mL and no clinical AIDS diagnosis prior to 30 days prior to entry into care) at 13 U.S. NA-ACCORD clinical cohorts who had a recorded CD4 at presentation during 2012–2018. We calculated cumulative incidence of timely ART (within 30 days of entry into care) using the Kaplan-Meier survival function by year of entry into care, with additional stratifications by the period of entry into care (2012-2015 vs. 2016-2018) and by race/ethnicity or geographic region. Discrete time-to-event models were fit to assess trends in timely ART by calendar year adjusted for age, sex, risk group, race/ethnicity, geographic region, AIDS diagnosis, history of alcohol or drug dependence/abuse, mental health diagnoses, and CD4 and VL at presentation, overall and stratified by the period of entry into care.

**Results:** Among 11,853 eligible treatment-naïve PWH, 48% were men who had sex with men, 14% were women, 45% Black, 15% Hispanic/Latino, 32% aged 18-29, and 7% aged ≥60 years. Cumulative incidence of timely ART increased from 42% in 2012 to 82% in 2018, with gains across race/ethnic groups and regions (Figure). In the multivariable model for 2012-2018, lower rates of timely ART were seen in Black than White PWH (adjusted hazard ratio [aHR] 0.89, 95% confidence interval [CI] 0.83–0.94). PWH living in the South than the West (aHR 0.78, CI 0.69–0.88), and PWH with a history of drug dependence/abuse also had higher rates of timely ART, but PWH in the Northeast had higher rates (aHR 1.37, CI 0.99–1.90) than PWH in the West region; drug dependence/abuse history remained associated with delayed ART (aHR 0.72, CI 0.61–0.85).

**Conclusion:** Timely ART has substantially improved in the United States since the release of DHHS universal treatment guidelines. Although race/ethnic and some geographic disparities in timely ART lessened, PWH with drug dependence/abuse diagnosis still had deficits, suggesting the need for additional support services for this population.

**105 GEOGRAPHIC DIFFERENCES IN TIME TO VIRAL SUPPRESSION IN THE DEEP SOUTH, 2012-2019**

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2. Louisiana Department of Health, New Orleans, LA, USA
3. Alabama Department of Public Health, Montgomery, AL, USA
4. Mississippi Department of Health, Jackson, MS, USA

**Background:** As outlined in the United States (US) Ending the HIV Epidemic initiative, achieving early and sustained viral suppression (VS) following diagnosis of HIV infection is critical to improving outcomes and reducing transmission. Dramatic geographic variability in time from HIV diagnosis to VS exists within the US including the Deep South, a region disproportionately affected by the domestic epidemic. Understanding drivers of this heterogeneity is essential to inform individual and population health approaches to ending the epidemic.

**Methods:** We conducted a retrospective, population-based cohort study of all persons ≥13 years with newly diagnosed HIV from 2012-2019 in Alabama (AL), Louisiana (LA), and Mississippi (MS), using data collected in the Enhanced HIV/AIDS Reporting System (eHARS), a standardized document-based surveillance...
DECREASED HIV DIAGNOSES AMONG MSM OF COLOR IN THRIVE-FUNDED JURISDICTIONS, 2014-2018

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Background: While the total number of HIV diagnoses decreased in the United States from 2014-2018, diagnoses increased among young men who have sex with men (MSM) of color. THRIVE was a demonstration project that funded jurisdictional grants to help provide comprehensive HIV prevention and care services for MSM of color. Twenty-eight Metropolitan Statistical Area (MSA) jurisdictions were eligible for THRIVE funding because they had the highest rates of HIV diagnoses among Black/African American, Hispanic, and/or Black MSM. This study evaluated trends in HIV diagnoses among MSM in jurisdictions awarded THRIVE funding compared to jurisdictions eligible for THRIVE but not awarded funding.

Methods: Data from the National HIV Surveillance System were analyzed to determine the number of HIV diagnoses from 2014-2018 among White, Black, and Hispanic/Latino MSM for: 1) THRIVE-eligible jurisdictions that were awarded THRIVE funding, 2) THRIVE-eligible jurisdictions that were not awarded THRIVE funding, and 3) overall for the United States and Puerto Rico. The Estimated Annual Percent Change (EAPC) and 95% confidence interval (CI) were used to evaluate trends for each of the three groups stratified by age group and race/ethnicity.

Results: From 2014-2018, 130,508 MSM were diagnosed with HIV infection in the U.S.; 8.4% were in THRIVE jurisdictions and 26% in unfunded THRIVE-eligible jurisdictions. During 2014-2018 in THRIVE jurisdictions, HIV diagnoses significantly decreased among Black MSM (EAPC -4.4 [95% CI -6.0, -2.7]) and White MSM (-9.4 [-12.1, -6.6]), but EAPC for Hispanics/Latino MSM was insignificant (-1.0 [-4.3, 2.4]). In comparison, HIV diagnoses in the unfunded THRIVE-eligible jurisdictions and the United States show White MSM experienced a decrease but to a lesser extent than THRIVE jurisdictions; Black MSM and Hispanic/Latino MSM did not see either a decline or an increase (Table). For most age groups, HIV diagnoses decreased in THRIVE jurisdictions among Black MSM, White MSM, and Hispanic/Latino MSM but increased in both unfunded THRIVE-eligible jurisdictions and the United States for Black MSM aged 25-34 years.

Conclusion: Findings suggest that successful implementation of HIV testing and PrEP in THRIVE contributed to decreases in HIV diagnoses among Black and White MSM. Barriers to HIV prevention for Hispanic/Latino MSM in THRIVE communities need to be understood to inform interventions for HIV prevention in this population.

Table. Estimated Annual Percent Change (EAPC) in HIV diagnoses among White, Black, and Hispanic/Latino MSM in U.S. Metropolitan Statistical Areas (MSAs) by total and stratified age groups and THRIVE funding, 2014-2018.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>THRIVE funded</th>
<th>THRIVE-eligible jurisdictions not awarded</th>
<th>Overall for United States and Puerto Rico</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>EAPC</td>
<td>95% CI</td>
<td>EAPC</td>
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<tr>
<td>25-34</td>
<td>-1.0</td>
<td>(-4.3, 2.4)</td>
<td>-1.0</td>
</tr>
<tr>
<td>35-44</td>
<td>-1.0</td>
<td>(-4.3, 2.4)</td>
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<td>45-54</td>
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<tr>
<td>55-64</td>
<td>-1.0</td>
<td>(-4.3, 2.4)</td>
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GENDER-AFFIRMING SURGERY ASSOCIATED WITH HIGH VIRAL SUPPRESSION AMONG TRANSGENDER PWH

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Background: Although HIV surveillance contains information on HIV outcomes among transgender people with HIV (TPWH), it does not include other important data, e.g., gender-affirming care, which may impact HIV outcomes such as viral suppression (VS). 79% of NYC TPWH are enrolled in Medicaid, and recent policy changes have extended coverage for gender-affirming healthcare. We matched Medicaid data with the NYC HIV Registry to ascertain the association of gender-affirming surgery and VS.

Methods: Because Medicaid claims do not specify transgender status, an algorithm using sex, diagnosis codes, and prescriptions was developed and applied to Medicaid claims from 2013-2017. This cohort was matched to the HIV Registry to identify which TPWH obtained gender-affirming surgery. We compared VS among TPWH in Medicaid who had surgery to TPWH who did not access Medicaid and cisgender women and men. We compared VS by type of surgery and examined trends in VS pre- and post-surgery.

Results: We identified 6,335 transgender persons in Medicaid in 2013-2017, 1,764 (28%) of whom were TPWH. 185 (10%) TPWH in Medicaid had gender-affirming surgery. They had higher VS at last lab and the greatest increase in VS over the five years (86.5%, 8.9% increase) compared to TPWH who did not access Medicaid (78.6%, 7.5% increase), cisgender women (82.3%, 8.7% increase), and cisgender men (84.1%, 6.9% increase). Those who had “bottom” surgery had the highest proportion suppressed (89%) compared to other types of surgery.
of surgery. VS increased pre-surgery, at least in part due to the common practice requiring viral suppression prior to surgery, and remained high over time (66.3% 2 years prior, 76.9% 1 year prior, 86.3% 1 year after (among everyone), and 87.7% 2 years after (among those who had surgery prior to 2017)).

**Conclusion:** Medicaid is a valuable source of data on transgender individuals and can complement data collected by HIV surveillance. While we cannot determine causality, it appears that preparing for gender-affirming surgery may be an important motivator in becoming virally suppressed. Moreover, it is associated with sustained high viral suppression, which is known to lead to improved survival and quality of life. Expanding Medicaid programs to include gender-affirming surgical care may be associated with better health outcomes among TPWH.

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Sophie Sembajwe, Andria Apostolou, Jeffrey McCollum, Azfar-E-Alam Siddiqi, Irene Hall, Jiannin Liu, Baohua Wu

**Background:** HIV diagnosis rates among American Indian/Alaska Natives (AI/AN) are higher than rates in whites and some minorities such as Asians. We evaluated HIV trends in diagnoses and mortality among AI/AN between 2014-2018.

**Methods:** We analyzed HIV surveillance data reported to CDC by state and local health departments focusing on the AI/AN population from 2014-2018. We focused on HIV diagnoses, prevalence of diagnosed cases and death rates per 100,000 AI/AN by age, sex, as well as Indian Health Service (IHS) geographic designations consisting of twelve physical areas of the United States: Alaska, Albuquerque, Bemidji, Billings, California, Great Plains, Nashville, Navajo, Oklahoma, Phoenix, Portland, and Tucson.

**Results:** Overall, from 2014 to 2018 the HIV diagnosis rate remained stable (2014: 7.6, 2018: 7.7 per 100,000). Increases were observed among those in the 13-24 years (8.2%) and 35-44 years (13.8%) age groups, with the latter having the highest percent increase among all age groups. Overall, the percentage of AI/AN living with diagnosed HIV increased 20.7% from 2014 to 2018. These increases were observed across almost all categories. Overall, the death rates from 2014-2018, decreased 31.4%. By IHS area, in 2018, the Navajo area experienced the highest HIV diagnosis rate per 100,000 population (13.9) followed closely by the Albuquerque (13.3) and Phoenix (12.7) areas. Nashville experienced the lowest HIV diagnosis rates during 2018 (1.8). In 2018, the majority of diagnoses across most IHS areas, were among men with HIV infection attributed to male-to-male sexual contact. In 2018, the Phoenix area had the highest rate per 100,000 of AI/AN persons living with HIV at 250.2, while Bemidji area had the lowest rate of AI/AN persons living with HIV at 44.0. In 2018, the Billings area experienced the highest death rates among AI/AN living with HIV, at 4.7 per 100,000 population. Bemidji, California, Nashville and Tucson areas reported no deaths in 2018.

**Conclusion:** The findings indicated that there have been increases in HIV diagnoses among AI/AN subgroups from 2014-2018, particularly in the 13-24 and 35-44 age groups. Furthermore, in 2018, the Navajo area experienced the largest burden of HIV diagnoses. In terms of HIV death rates, there have been general decreases across the board for AI/AN populations nationally and in IHS-area-specific jurisdictions.
RCT OF AN ONLINE MENTAL HEALTH INTERVENTION AMONG OLDER PLWH DURING COVID-19 PANDEMIC

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Background: Older adults (≥50 y) living with HIV (OALWH) may experience elevated levels of depression, anxiety, and loneliness. Online mindfulness lessons have the potential to ameliorate these problems and enhance access, especially during the COVID-19 pandemic. The objective of this randomized controlled trial was to determine the effectiveness of online mindfulness lessons in reducing feelings of depression, anxiety, and loneliness among OALWH.

Methods: The study was conducted between May and August 2020. Individuals with any degree of self-reported loneliness at baseline were eligible to participate. Outcomes of interest included depression measured using the Center for Epidemiologic Studies Depression Scale (CES-D-10), anxiety measured using the Generalized Anxiety Disorder (GAD-7), and loneliness measured using both the Three-item Loneliness Scale (3IL) and a Daily Diary that asked “How lonely do you feel today?” Two sample t-tests were used to compare group scores at follow-up.

Results: Of 214 participants who were randomized, the mean (SD) age was 60.4 (5.9) years, 89% were male, 69% were white, and 74% were gay or lesbian. Of 214 participants who were randomized, the mean (SD) age was 60.4 (5.9) years, 89% were male, 69% were white, and 74% were gay or lesbian. Outcomes of interest included depression measured using the Center for Epidemiologic Studies Depression Scale (CES-D-10), anxiety measured using the Generalized Anxiety Disorder (GAD-7), and loneliness measured using both the Three-item Loneliness Scale (3IL) and a Daily Diary that asked “How lonely do you feel today?” Two sample t-tests were used to compare group scores at follow-up.

Conclusion: This randomized controlled trial is the first to show that a series of brief, online mindfulness audio lessons improves mental health outcomes among OALWH who report some degree of loneliness. For many patients, this intervention may offer emotional relief, particularly with regard to depression and anxiety, even in the face of the COVID-19 pandemic.
SEVERE COVID-19 IS FUELED BY DISRUPTED GUT BARRIER INTEGRITY
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Background: A disruption of the crosstalk between gut and lung has been implicated as a driver of severity during several respiratory-related diseases. Lung injury causes systemic inflammation, which disrupts gut barrier integrity, increasing the permeability to gut microbes and their products. This exacerbates inflammation, resulting in positive feedback. We applied a multi-omic systems biology approach to investigate the potential link between loss of gut barrier integrity and Coronavirus disease 2019 (COVID-19) severity.
Methods: We analyzed plasma samples from age and gender-matched COVID-19 patients (n=60) with varying disease severity (mild, moderate, and severe) and 20 SARS-CoV2 negative controls. We measured markers and drivers of tight junction permeability and microbial translocation using ELISA; inflammation and immune activation markers using ELISA and multiplex cytokine arrays; untargeted metabolomic and lipidomic analyses using mass spectrometry; and plasma glycymes using capillary electrophoresis and lectin microarray. False discovery rate (FDR) was calculated to account for multiple comparisons.
Results: Our data indicate, first, that severe COVID-19 is associated with a dramatic increase in the level of zonulin (FDR<0.00001), the only known physiological driver of intestinal tight junction permeability. This increased permeability associated with translocation of both bacterial (LPS binding protein (LBP) levels) and fungal (β-glucan levels) products into blood (FDR<0.01). The degree of intestinal permeability and microbial translocation strongly correlated with increased systemic inflammation (correlations with IL-6 and other inflammatory cytokines and markers) (FDR<0.05). Second, levels of metabolic and lipidomic markers of gut and gut microbiota functionality including citrulline (a marker of healthy gut; decreased), succinic acid, and tryptophan catabolism metabolites (markers of microbial dysbiosis; increased) were disrupted during severe COVID-19 (FDR<0.05). Finally, the gut microbiome is known to release enzymes that degrade plasma glycans, which regulate inflammation and complement activation. Indeed, severe COVID-19 was associated with loss of the anti-complement activation galactosylated glycans from plasma and IgG glycoproteins (FDR<0.05).
Conclusion: Our data provide multiple layers of evidence that a previously unappreciated factor with significant clinical implications, disruption in gut barrier integrity, is a potential force that contributes to COVID-19 severity.
SARS-CoV-2 persists in intestinal enterocytes up to 7 months after symptom resolution

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Background: Host proteins ACE-2 and TMPRSS2 facilitate SARS-CoV-2 infection and are expressed in the lungs as well as the intestinal tract, particularly in the small bowel. Gastrointestinal symptoms represent the most common extrapulmonary manifestation of COVID-19. Viral RNA has been isolated from fecal samples from COVID-19 patients, where it can persist longer than detection in nasopharyngeal swabs. While SARS-CoV-2 infection of enterocytes has been demonstrated in vitro, little is known about its persistence in vivo.

Methods: Small intestinal biopsies from patients who underwent clinically indicated endoscopic procedures after a positive SARS-CoV-2 nasopharyngeal swab (n=27) or were found to have positive serology (n=2) were analyzed by immunofluorescence (IF) (n=25) and electron microscopy (EM) (n=14) for the presence of SARS-CoV-2 viral RNA. Clinical details were also collected.

Results: Sixteen of 29 patients had detectable SARS-CoV-2 antigen by either IF or EM (Figure 1A). Virus was restricted to the epithelium and patchy in distribution. Virus was detected as soon as 15 days after symptom onset and persisted up to 6 months after symptom resolution. Five patients were nasopharyngeal swab positive at the time of procedure and, of these, 4 had detectable antigen on biopsy. Despite the presence of virus, only 9/16 patients had any signs of inflammation on histology, and when present, this was mild. In two patients where virus was present at 3 months and 4 months, additional biopsies were obtained at 7 months and 6 months, respectively. Viral antigen was persistently detected in both patients and both patients were nasopharyngeal swab negative for all procedures. Interestingly, only 37.5% (6 of 16) of patients with virus detected in the small bowel had GI symptoms (diarrhea, nausea or vomiting) during their acute COVID-19 illness as compared to 46.1% (6/13) of patients where no virus could be detected in the intestines.

Conclusion: SARS-CoV-2 infects enterocytes in humans in vivo and can persist in the intestines up to 7 months following symptom resolution. This persistence is not associated with an overt inflammatory infiltrate and does not appear to correlate with presence of GI symptoms in the acute COVID-19 setting.
CHARACTERIZATION AND EPITOPE MAPPING OF SARS-CoV-2–SPECIFIC T CELLS
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2Oregon Health and Sciences University, Portland, OR, USA
3Emory University, Atlanta, GA, USA, 4Oregon Health and Sciences University, Portland, OR, USA
Background: The role that CD4+ and CD8+ T cells play in the protection from and disease severity of COVID-19 is not completely understood. A better understanding of T cell function and the epitopes that they target will be invaluable in the development of the next generation of vaccines and therapeutics. To better understand the role of T cells, we characterized the frequency, effector functions and phenotype of SARS-CoV-2–specific CD4+ and CD8+ T cells in a cohort of patients who recovered from COVID-19, and identified multiple peptides that contain T cell epitopes within the Spike protein (S), Nucleocapsid protein (N) and Membrane protein (M).

Methods: The frequency and phenotype of SARS-CoV-2–specific T cells from convalescent patients with mild or moderate disease (n=27, 25 to 92 days post-symptom onset) were determined by polychromatic flow cytometry and intracellular cytokine staining (ICS). Cells were stimulated for 6 hours with peptide pools corresponding to S, N and M. Cytokine production, memory phenotype, chemokine receptor expression and PD-1 expression were analyzed. For a subset of individuals (n = 19 for S; n=14 for N and M), IFNg ELISpot assays and peptide matrices were utilized to identify peptides that contain T cell epitopes.

Results: CD4+ T cell responses to S, N and/or M were detected in almost all donors by ICS and were predominantly a Th1-type response as determined by cytokine production (IFNg, IL-2 or TNF) and expression of CXCR3. A majority of the antigen–specific CD4+ cells were found in the effector memory compartment. Although less robust than the CD4+ T cell response, antigen–specific CD8+ T cells were detected in a majority of donors, were found within the effector memory compartments and displayed modest PD-1 upregulation. Multiple peptides that contain T cell epitopes were identified by IFNg ELISPOT (Figure 1). Some of the most commonly identified peptides include S42 (amino acids 165-179; 7/19 donors), S205 (a.a. 817-831; 10/19 donors), N83 (a.a. 329-343; 7/14 donors), M37 (a.a. 145-159; 8/14 donors) and M45 (a.a. 177-191; 10/14 donors).

Conclusion: These data suggest that T cells that target S, N and M play an important role in the immune response to SARS-CoV-2 and should be considered in future vaccine development. Further studies such as transcriptomic analysis and the TCR usage in longitudinal samples will provide a better understanding of epitope–specific T cells and their longevity.

118 BARICITINIB LOWERS INFLAMMATION AND PATHOLOGY IN SARS-CoV-2–INFECTED RHESUS MACAQUES
Timothy Hoang1, Maria Pino1, Arun Boddapati1, Elise Viox1, Carly E. Starke1, Amit Upadhyay1, Sanjeev Gumber1, Susan Pereira Ribeiro1, Rafiek-Pierre Sekaly1, Rebecca Levit1, Jacob Estes1, Thomas H. Vanderford2, Raymond Schnaizer3, Steven Bossinger1, Mirko Paiardini1
1Emory University, Atlanta, GA, USA, 2Oregon Health and Sciences University, Portland, OR, USA
Background: The emergence of SARS-CoV-2 and COVID-19 pandemic has placed an excessive burden on public and private healthcare systems with over 1,400,000 deaths worldwide. Thus, therapeutics aimed at mitigating disease severity are urgently needed. Immunological features of COVID-19 progression include an influx of innate and adaptive immune cells to the lung, with severe cases having elevated levels of pro-inflammatory cytokines and chemokines. Baricitinib is an oral, selective inhibitor of JAK 1/2 with potent anti-inflammatory activity approved for patients with moderate to severe active rheumatoid arthritis and predicted to have anti-SARS-CoV-2 effects based on silico modeling.

Methods: 8 rhesus macaques (RMs) were infected with 1.1x10^6 PFU SARS-CoV-2; at 2 days post infection (dpi), 4 of the 8 RMs began daily baricitinib treatment (4 mg/day). Nasal and throat swabs were collected daily for viral load; longitudinal blood and bronchoalveolar lavage (BAL) samples were collected for viral load, flow cytometry, cytokines and RNAseq analysis and at 10/11 dpi all RMs were euthanized for pathological analyses.

Results: Baricitinib was found in plasma and in the lungs of all treated RMs and was safe and well tolerated. Viral replication dynamics measured from nasal and throat swabs, BAL and lung at necropsy were not reduced with baricitinib. Innate Type-I IFN antiviral responses and adaptive SARS-CoV-2–specific T-cell responses remained similar between the two groups. RMs treated with baricitinib showed reduced inflammation (ferritin, CRP, histology), T cell immune activation and proliferation, neutrophil NETosis activity, and lung pathology, with decreased type 2 pneumocyte hyperplasia, peribronchiolar hyperplasia, and inflammatory cell infiltration. Importantly, baricitinib treated RMs had a rapid and remarkably potent suppression of alveolar macrophage production of cytokines (IL-6, TNFa, IL-10, IL-1b and IFNb1) and chemokines (CCL4L1, CXCL10, CXCL3 and CXCL8) responsible for a pro-inflammatory environment and for the recruitment of neutrophil and pro-inflammatory monocytes. Additionally, we identified that a population of MARCO+ macrophages are the primary producers of pro-inflammatory cytokines and are reduced in the lungs of baricitinib treated animals.

Conclusion: These data provide rationale and mechanistic insight for the use of baricitinib as a frontline therapeutic to reduce systemic inflammation induced following SARS-CoV-2 infection.
ONE DOSE OF COVID-19 mRNA VACCINE IN SARS-CoV-2–EXPERIENCED PEOPLE MAY BE SUFFICIENT

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Background: Understanding if single doses of SARS-CoV-2 mRNA vaccines in SARS-CoV-2–experienced people are fully protective is a public health priority. This study measured immune responses before and after mRNA vaccine in people with or without histories of COVID-19.

Methods: Specimens were collected from participants before and 6–14 days after doses 1 and 2. Humoral assays included an S1-specific IgG ELISA and a live-virus microneutralization assay (MN) vs the original SARS-CoV-2 USA-WA1/2020 strain. ELISpot assays and 36-color spectral analysis flow cytometry assessed B- and T-cell responses.

Results: 32 adults received Pfizer BioNTech vaccine and 1 received Moderna vaccine. 14 had a history of COVID-19 (median age 41, 71% female, 10 with 3/20 and 2 with 12/20 illness onset, 2 asymptomatic). 19 were SARS-CoV-2–naïve (median age 39, 47% female). S1-specific IgG/M ASC were detected readily by ELISpot 6–14 days after dose 1 and were higher in SARS-CoV-2–experienced (median: 200) than -naïve (median: 27) subjects; after dose 2, the converse was observed (medians 53 vs 293). By flow cytometry, T cell activation was broadly observed 6–14 days after 1st vaccination, with increases in CD4+ or CD8+ T cells expressing CD38 and Ki67 (CD4: median fold-changes 1.6 for SARS-CoV-2–experienced and 1.8 for -naïve; CD8: 3.1 and 2.2). S1-specific IgG was present at baseline in experienced subjects (median: 6320), peaked at 6–14 days post-dose 1 (median: 169000), and wasn’t boosted by dose 2 (panel A). In naïve participants, S1-specific IgG was not present at baseline, low at day 6–14 (median: 66), higher at day 21 (median: 27000), and boosted by dose 2 (median: 188000). Interestingly, by 6–14 days after dose 2, experienced and naïve subjects had similar S1-specific IgG titers. The MN titers followed a similar pattern (panel B): in experienced subjects, striking increases after dose 1 (median: 9860) but no boosting by dose 2; in naïve subjects, no neutralization was observed at 6–14 days, low titers were present at 21 days post-dose 1 (median: 43), with boosting after dose 2 (median: 513).

Conclusion: People with a history of SARS-CoV-2 infection who received a single dose of mRNA vaccine produced binding and neutralizing antibody titers at 6–14 days that were similar to, or higher than, titers in SARS-CoV-2–naïve people who had received 2 doses. Their titers were not boosted by a second dose. These findings support a hypothesis that SARS-CoV-2–experienced people may require only a single dose of mRNA vaccine.

SARS-CoV-2 RECRUITS A HAEM METABOLITE TO EVADE ANTIBODY IMMUNITY

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Background: Understanding antibody immunity to SARS-CoV-2 and how the virus evades it is of critical importance in the fight against COVID-19. Our best hope of ending the pandemic is antibody-inducing vaccination, yet the precise targets and indeed protective capacity of antibodies remain incompletely defined. The coronaviral spike is the dominant viral antigen and the target of neutralizing antibodies. We discovered neutralizing epitopes located on the distal face of the SARS-CoV-2 spike N-terminal domain (NTD). Remarkably, instead of glycosylation, the virus uses a surface-exposed loop to restrict the access to this patch, and the gate is controlled through recruitment and dissociation of a metabolite.

Methods: Using cryo-electron microscopy and X-ray crystallography we mapped a tetapyrrole binding site to a deep cleft on the spike N-terminal domain (NTD, Fig. 1) and characterized structural features of a neutralizing epitope controlled by metabolite dissociation.

Results: We show that SARS-CoV-2 spike binds biliverdin and bilirubin, the tetapyrrole products of haem metabolism, with nanomolar affinity in a pH-sensitive manner. At physiological concentrations, biliverdin significantly dampened the reactivity of SARS-CoV-2 spike with immune sera and inhibited a subset of neutralizing antibodies. Access to the tetapyrrole-sensitive epitope is gated by a flexible loop on the distal face of the NTD. Accompanied by profound conformational changes in the NTD, antibody binding requires relocation of the gating loop, which folds into the cleft vacated by the metabolite.

Conclusion: It is well-established that viruses employ extensive glycosylation of their envelopes to shield antibody epitopes. Compared to glycosylation, epitope masking via metabolite recruitment has the advantage of reversibility. For instance, pH-dependence of the spike-tetapyrrole interaction potentially allows dissociation within the late endosomal compartment. In summary, our results indicate that the virus co-opt the haem metabolite for the evasion of humoral immunity via allosteric shielding of a sensitive epitope and demonstrate the remarkable structural plasticity of the NTD.

BAMLANIVIMAB PREVENTS COVID-19 MORBIDITY AND MORTALITY IN NURSING-HOME SETTING

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1Institute of Global Health and Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 2Eli Lilly and Company, Indianapolis, IN, USA; 3New York University Langone Vaccine Center, Division of Infectious Diseases and Immunology, New York University Grossman School of Medicine, New York, NY, USA; 4University of Illinois College of Medicine, Chicago, IL, USA; 5Vaccine Research Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; 6Indiana University School of Medicine, Gary, IN, USA

Background: The COVID-19 pandemic has disproportionately affected residents of skilled nursing and assisted living facilities. Interventions are urgently needed to protect this vulnerable population. Bamlanivimab is a potent neutralizing monoclonal antibody that binds the receptor-binding domain of the spike protein of SARS-CoV-2. This study evaluates the safety and efficacy of bamlanivimab in preventing COVID-19.

Methods: BLAZE-2 is a Phase 3, randomized, double-blind, placebo-controlled, single-dose study that enrolled residents and staff at skilled nursing and assisted living facilities reporting at least one confirmed SARS-CoV-2 case. Eligible participants received bamlanivimab (4200 mg) or placebo intravenously. Nasal swabs were collected at baseline and weekly through day 57 to determine SARS-CoV-2 infection status via reverse transcriptase polymerase chain reaction (RT-PCR). COVID-19-related symptoms and signs were recorded daily. The primary analysis prevention population included participants negative at
BAMLANIVIMAB+ETSEVIMAB FOR TREATMENT OF COVID-19 IN HIGH-RISK AMBULATORY PATIENTS

Michael Dougall1, Ajay Nirula2, Robert L. Gottlieb1, Masoud Azizad1, Bharat Mocherla1, Peter Chen1, Gregory Huhn1, Andrew C. Adams2, Andrew E. Schade3, Janelle Sabo3, Dipak R. Patel4, Paul Klekotka2, Lei Shier5, Daniel M. Skovronsky4, for BLAZE-1 Investigators

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Background: Patients with underlying medical conditions have a greater risk of developing severe COVID-19. Unlike vaccine-derived immunity which develops over time, administration of neutralizing monoclonal antibodies is an immediate, passive humoral immunotherapy, with the potential to reduce disease progression, emergency room visits, hospitalizations, and death.

Methods: In this phase 3 portion of the BLAZE-1 trial, a high-risk ambulatory cohort of 1035 patients with mild-to-moderate COVID-19 were randomly assigned 1:1 to receive a single intravenous infusion of a neutralizing monoclonal antibody combination treatment consisting of 2800mg bamlanivimab+2800mg etesevimab together, or placebo, within 3 days of sore throat and fever onset and within 24 hours of nasopharyngeal swab specimen collection or symptom onset in those who tested negative for SARS-CoV-2 by RT-PCR within 96 hours of their household member testing positive. The primary outcome was overall patient clinical status, measured by the proportion of patients who experienced COVID-19-related hospitalization or death by any cause by Day 29.

Results: 1035 patients were randomized and infused (mean age 50.3 years [16.8], female 52%). A 70% reduction in COVID-19-related hospitalization and death by any cause by Day 29 was observed in patients who received the bamlanivimab+etesevimab combination treatment (Δ[95% CI]=−1.20[−1.46,−0.94];p<0.00000001). The median time to sustained symptom resolution was shorter for those who received the combination treatment (days [95% CI]=4[3.0,4.0]) compared to those who received placebo (days [95% CI]=10[9.0,11.0];p=0.007). Similar rates of adverse events were observed between placebo (60/517 arm total) and combination treatment groups (69/518[13.3%]).

Conclusion: Bamlanivimab was highly effective in reducing the incidence of symptomatic COVID-19 and SARS-CoV-2 infection and was well tolerated. These findings demonstrate the potential beneficial impact of bamlanivimab use on COVID-19 morbidity and mortality among skilled nursing facility residents.

122 BAMLANIVIMAB + ETSEVIMAB FOR TREATMENT OF COVID-19 IN HIGH-RISK AMBULATORY PATIENTS

123 CASIRIVIMAB WITH IMDEVIMAB ANTIBODY COCKTAIL FOR COVID-19 PREVENTION: INTERIM RESULTS

Meagan P. O’Brien1, Eduardo Forleo Neto1, Kuo-Chen Chen1, Flonza Isa1, Ingeborg Heirman1, Neena Sarkar2, Divya Ramesh1, Myron S. Cohen3, Ruanne Barnabas1, Christopher B. Hurt4, Dan H. Barouch1, Katharine J. Bar1, Gary Herman5, George D. Yancopoulos6, David M. Weinreich7, Regeneron Pharmaceuticals, Inc., Sarytown, NY, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3University of Washington, Seattle, WA, USA, 4Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA, 5University of Pennsylvania, Philadelphia, PA, USA

Background: Passive immunization has a long history for infection prevention following exposure. We report results of a descriptive interim analysis from a study of an antibody “cocktail” of casirivimab with imdevimab (cas/imdev; formerly REGN-COVID) designed to bind non-competing epitopes of the spike protein, as a potential passive vaccine for the prevention of COVID-19 in people at risk of infection from household contact.

Methods: In this ongoing Phase 3 study, asymptomatic participants exposed to a COVID-19–infected household member were randomized 1:1 to placebo or 1200 mg cas/imdev (600 mg of each antibody administered subcutaneously) within 96 hours of their household member testing positive. The analysis included participants who tested negative for SARS-CoV-2 by nasal, saliva, or nasopharyngeal swab and who were seronegative to SARS-CoV-2 antibodies at baseline. The proportion of participants who developed an RT-PCR-confirmed SARS-CoV-2 infection (asymptomatic or symptomatic) during the 1-month efficacy assessment period was summarized.

Results: Initial results from the first evaluable 223 placebo and 186 cas/imdev participants who completed ≥29 days of the study are reported. Reduction in PCR-positive asymptomatic disease was 100% (0/186 cas/imdev vs 8/223 placebo; OR 0.00 [CI 0.00, 0.69]). Reduction in any PCR-positive infection
124 POTENT NEUTRALIZING ANTIBODIES FOR SEVERE COVID-19: A RANDOMIZED CLINICAL TRIAL

Carlijn Jordans, Arvind Gharbharan, Corine H. Geurts van Kessel, Jeroen J. Van Kampen, Barry Rockx, Bart Haagmans, Francis Swaneveld, Yvonne Mueller, Peter Katsikis, Marion Koopmans, Bart J. Rijnders, Casper Roks, for the CONCOVID Trial Network

Erasmus University Medical Center, Rotterdam, Netherlands

Background: Convalescent plasma could be an inexpensive and widely available drug for COVID-19 patients. Reports on its effectiveness are inconclusive. We collected convalescent plasma with high titers of neutralizing anti-SARS-CoV-2 antibodies effectively blocking SARS-CoV-2 infection and assessed their clinical and viro-immunological responses in COVID-19 patients with severe disease.

Methods: In a multicentre open-label randomized clinical trial in 14 secondary and academic hospitals in the Netherlands, included patients were admitted for COVID-19 with SARS-CoV-2 detected by PCR and not on mechanical ventilation for >96hours. Convalescent plasma donors were selected based on SARS-CoV-2 plaque reduction neutralization test (PRNT50). Primary outcome was day 60 mortality. Secondary outcomes were disease severity, inflammatory and virological markers.

Results: Included patients were 72% male, median 63 years (IQR 56-74) and with median 10 days of symptoms (IQR 6-15) at inclusion when they were randomized to convalescent plasma or standard of care. We found no significant difference in mortality at day 60 by using 300mL of convalescent plasma on viral clearance in the respiratory tract, anti-SARS-CoV-2 antibody cocktail prevented symptomatic infection, reduced overall infection, and decreased viral load and duration of viral RNA detection.

Conclusion: In this descriptive interim analysis of participants at risk of SARS-CoV-2 infection from household transmission, a subcutaneous dose of the cas/imdev antibody cocktail prevented symptomatic infection, reduced overall infection, and decreased viral load and duration of viral RNA detection.

125 ESTIMATING WITHIN-HOST RO FOR SARS-COV-2 AND IMPLICATIONS FOR ANTIVIRAL THERAPY

Ruian Ke, Ruy M. Ribeiro, Alan S. Perelson

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Background: The within-host reproductive number R0 is an important parameter to predict the minimum antiviral efficacy needed to suppress viral infection. However, this parameter has not been well quantified for SARS-CoV-2. This is because accurate estimation of this quantity requires longitudinal viral load measurements during the initial phase of infection, when the virus population expands before the viral load peak; yet, most available measurements are made after the viral load peak.

Methods: We constructed viral dynamic models to describe a set of longitudinal viral load data from a study where individuals were tested frequently such that viral loads during the viral expansion phase were measured. We fit multiple models to data from a total of 42 infected individuals (14 symptomatic and 28 asymptomatic) to estimate R0 and used a model linking within-host viral load to the infectiousness of a person to evaluate the infectiousness of asymptomatic individuals compared to symptomatic individuals.

Results: We estimated that the within-host R0 is between 8-16 across the 48 individuals. This suggests that antiviral efficacy has to be greater than 95% to suppress virus infection in a majority of individuals. The estimated R0 in asymptomatic individuals is lower than in symptomatic individuals (mean 10.0 vs. 13.8; p-value<0.0001). Our model suggests there exists large heterogeneity in infectiousness among individuals, and asymptomatic individuals may be on average 15% less infectious than symptomatic individuals (p-value=0.02), not considering isolation measures.

Conclusion: An antiviral efficacy of 95% or more is needed to suppress viral infection in most infected individuals. Asymptomatic individuals may be slightly less infectious than symptomatic individuals.
individuals. 4/6 participants in the 200-mg group (part 1) developed RAMs on Day 11; 1 of these 4 developed phenotypic resistance. No RAMs were observed at any dose in part 2. No deaths were reported. Two non-drug-related serious adverse events (AEs) of congestive cardiomyopathy and anal abscess occurred. No AEs led to discontinuation. AEs were reported by 22 (65%) participants, with the most common being headache (n=4). Drug-related gastrointestinal AEs, including diarrhea (n=3), abdominal pain (n=2), and vomiting (n=2), occurred in 6 (18%) participants. All AEs and serious AEs were mild to moderate in intensity, except for 1 participant who developed congestive cardiomyopathy and myocarditis (both g)

Conclusion: This short-term monotherapy study established a dose—antiviral response relationship. Regardless of dosing duration, GS-254 150- and 200-mg doses demonstrated greatest declines in plasma HIV-1 RNA. No safety or tolerability concerns were noted. These results support the ongoing phase Ib study (ClinicalTrials.gov identifier: NCT04492126).

Table. Change From Baseline to Study Visit 6 and Nadir in Plasma HIV-1 RNA

<table>
<thead>
<tr>
<th>Plasma HIV-1 RNA (log10, mean (SD))</th>
<th>Part 1</th>
<th>Part 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS'254 10 mg (NNRTI)</td>
<td>4.19 (0.31)</td>
<td>4.92 (0.47)</td>
</tr>
<tr>
<td>GS'254 200 mg (NNRTI)</td>
<td>4.24 (0.417)</td>
<td>4.87 (0.233)</td>
</tr>
<tr>
<td>Placebo (Vehicle)</td>
<td>4.43 (0.510)</td>
<td>4.53 (0.577)</td>
</tr>
<tr>
<td>GS'254 40 mg (NNRTI)</td>
<td>4.01 (0.79)</td>
<td>4.01 (0.79)</td>
</tr>
<tr>
<td>GS'254 80 mg (NNRTI)</td>
<td>4.01 (0.79)</td>
<td>4.01 (0.79)</td>
</tr>
<tr>
<td>GS'254 140 mg (NNRTI)</td>
<td>4.01 (0.79)</td>
<td>4.01 (0.79)</td>
</tr>
<tr>
<td>Placebo (Vehicle)</td>
<td>4.01 (0.79)</td>
<td>4.01 (0.79)</td>
</tr>
</tbody>
</table>

Conclusions:

127 POTENT ANTIVIRAL ACTIVITY OF LENACAPAVIR IN PHASE 2/3 IN HEAVILY ART-EXPERTISED PWH

Sorana Segal-Maure1, Antonella Castagna2, Mezegebe Berhe3, Gary Richmond4, Peter J. Ruane4, Gary J. Sinclair5, Krittatchao Siripassorn6, Ya-Pei Liu6, Nicolas Margot1, Hadas Dvory-Sobol7, Robert H. Hyland8, Martin Rhee9, Jared Baeten1, Diana Brainard1, Edwin DeJesus9

1New York-Presbyterian Queens, Flushing, NY, USA, 2Ospedale San Raffaele, Milano, Italy, 3Texas Infectious Diseases Consultants, Dallas, TX, USA, 4Gilead Sciences, Inc, Foster City, CA, USA, 5Rhein Clinical Research Group, Los Angeles, CA, USA, 6Prion Health North Texas, Dallas, TX, USA, 7Ramamurthy Infectious Diseases Institute, Bangkok, Thailand, 8Gilead Sciences, Inc, Foster City, CA, USA, 9Orlando Immunology Center, Orlando, FL, USA

Background:

Lenacapavir (LEN, GS-6207), the long-acting first-in-class HIV capsid inhibitor, is in clinical development for the treatment and prevention of HIV-1 infection. With its novel mechanism of action, LEN is fully active in vitro against HIV-1 strains resistant to the major antiretroviral (ARV) classes.

Methods:

We conducted a Phase 2/3, randomized, double-blind, placebo (PBO)-controlled study in heavily treatment-experienced (HTE) people with HIV (PWH) failing their current regimen with HIV-1 RNA (VL) ≥ 400 c/mL and documented resistance to ≥ 2 agents from ≥ 3 of the 4 major ARV classes. Participants were randomized (2:1) to add LEN or PBO to their failing regimen for 2 weeks. During this functional monotherapy period, LEN or PBO was given orally (600 mg on Day [D] 1 and 2 and 300 mg on D8). The primary efficacy endpoint was the proportion of participants with at least 0.5 log10 c/mL decline in VL by D15. At D15, those on oral LEN received subcutaneous (SC) LEN 927 mg (q6month), while those on PBO started the LEN 2-week oral lead-in, followed by q6month SC. All participants initiated an investigator-selected, optimized background regimen (OBR) at D15. Here we report complete data for the LEN+OBR period. At D15, those on oral LEN received subcutaneous (SC) LEN 927 mg (q6month), while those on PBO started the LEN 2-week oral lead-in, followed by q6month SC. All participants initiated an investigator-selected, optimized background regimen (OBR) at D15. Here we report complete data for the LEN+OBR period.

Results:

Participants were randomized: 28% were female and 46% Black. Median age was 54 years. Mean baseline VL was 4.27 log10 c/mL. At D15, 88% of participants on LEN (21 of 24) had at least 0.5 log10 c/mL decline compared to 17% on PBO (2 of 12) (difference: 75%, 95% CI 35 to 90%, p<0.0001). The median (range) change in VL (log10 c/mL) was -2.00 (-3.29 to -0.29) vs -0.08 (-1.93 to 0.31). During the LEN + OBR period at 4 weeks after SC dosing, 58% (21 of 36) had VL <50 c/mL. One participant with no fully active agent in OBR background regimen (OBR) at D15. Here we report complete data for the LEN+OBR period.

One participant with no fully active agent in OBR background regimen (OBR) at D15. Here we report complete data for the LEN+OBR period.

Conclusion:

128 ACTIVITY AND RESISTANCE CHARACTERIZATION OF THE HIV CAPSID INHIBITOR LENACAPAVIR

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Background:

Lenacapavir (LEN) in vitro resistance selections identified resistance associated mutations (RAMs) in HIV-1 capsid (CA) associated with reduced susceptibility (L56I, M66L, Q67H, K70N, Y74F, T74R), and T110RN. Characterization of mutants in single-cycle (SC) assays indicated that most of these RAMs were associated with reduced replication capacity (RC). We studied LEN antiviral activity in isolates containing LEN-RAMs in SC and multi-cycle (MC) assays to determine their impact on RC across several assay formats and to explore their potential cross-resistance with protease inhibitors (PIs). In addition, the activity of LEN was determined in previously untested HIV-1 subtypes.

Methods:

Mutations were inserted in the pXILX plasmic clone by site-directed mutagenesis; replicative viruses were generated by transfection. Viruses (6 single- and 4 double-mutants) were tested in 2 different MC formats to determine susceptibility to LEN and controls. Mutated sequences were used to generate SC test vectors that were tested in a Gag-Pro assay, along with isolates with diverse HIV-1 subtypes (A, B, C, B, BF, C, D, G, and H) (Monogram).

Results:

Antiviral activity and susceptibility in the SC assay confirmed the previously observed data, with Q67H leading to a 4.6-fold reduction in susceptibility to LEN and 58% and RC in comparison with wild-type (WT); M66I showed a >2000-fold reduction in susceptibility and 1.5% RC. Although LEN susceptibility data were obtained for all 10 mutants in the SC assay, in the more restrictive MC assay, data were only obtained for 6 of the 10 mutants, with 4 lacking measurable infectivity in the assay, particularly the M66I and M66L + Q67H mutants. Overall, LEN susceptibility values in the MC assay were similar to those in the SC assay. Susceptibility to the PIs darunavir and atazanavir was unchanged from WT in the SC and MC assays. LEN showed similar potency across diverse subtypes in the SC assay, with EC50 values (range: 124 - 357 pM) on par with that of the WT control (290 pM).

Conclusion:

Susceptibility to LEN in single-cycle and multi-cycle assay formats has expanded our understanding of LEN resistance in vitro and confirmed the decreased RC of the mutant viruses. In addition, mutants with resistance to LEN were found not cross-resistant to PIs, and LEN susceptibility was similar across isolates with diverse subtypes. These data emphasize the beneficial profile of LEN as a potential treatment option for people living with HIV.

129 RESISTANCE PROFILE OF MK-8507, A NOVEL NNRTI SUITABLE FOR WEEKLY ORAL HIV TREATMENT

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1Merck & Co Inc, West Point, PA, USA

Background:

MK-8507 is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) in clinical development as a once-weekly oral treatment for HIV-1 infection. To support the development of MK-8507, in vitro assays were performed to assess its virologic profile and compare its activity to clinically approved NNRTIs.

Methods:

Half-maximal inhibitory concentration (IC50) of MK-8507 against wild-type subtype B (WT) HIV-1 was assessed in a multiple cycle assay. Activity against clinical variants representing multiple HIV-1 subtypes was determined using the PhenoSense® assay. MK-8507 was evaluated in two-drug combination antiviral and cytotoxicity assays with each of 17 antiviral agents across mechanistic classes. Viral resistance selection studies with escalating concentrations of MK-8507 were conducted on cells infected with HIV-1 subtype A, B, or C to determine pathways to MK-8507 resistance. Antiviral activity of MK-8507 on common NNRTI resistance-associated variants and a panel of variants that emerged under MK-8507 selective pressure was determined in multiple cycle assays. Activity against a panel of clinical NNRTI resistance-associated variants was determined by PhenoSense®.
RIFAPENTINE +/- MOXIFLOXACIN FOR PULMONARY TUBERCULOSIS IN PEOPLE WITH HIV

April Pettit1, Payam Mahie2, Patrick P. Phillips3, Andrew Vernon4, Ekaterina Kurbatova5, Rodney Dawson6, Ian Sanne7, Ziyaad Waja8, Lerato Mohapi9, Wadzanai Samanaka10, John Johnson11, Susan Dormann12, Richard E. Chaisson13, Susan Swindells14, for the TBTC Study Group 31/ACTG S349 study team
1Vanderbilt University, Nashville, TN, USA, 2University of California San Francisco, San Francisco, CA, USA, 3Centers for Disease Control and Prevention, Atlanta, GA, USA, 4University of Cape Town, Cape Town, South Africa, 5University of Witwatersrand, Johannesburg, South Africa, 6Perinatal HIV Research Unit, Soweto, South Africa, 7University of Zimbabwe, Harare, Zimbabwe, 8Case Western Reserve University, Cleveland, OH, USA, 9Medical University of South Carolina, Charleston, SC, USA, 10Johns Hopkins University, Baltimore, MD, 11University of Nebraska Medical Center, Omaha, NE, USA

Background: Daily regimens with rifapentine (RPT) and moxifloxacin (MOX) have potent antimicrobial activity that may allow treatment shortening for drug-susceptible pulmonary tuberculosis (TB).

Methods: TB Trials Consortium Study 31/ACTG A3349 is an international, randomized, open-label, phase 3 non-inferiority (NI) trial comparing two 4-month RPT-based regimens with the 6-month control regimen (NI margin 6.6%). One 4-month regimen replaced rifampin with RPT 1200mg (RPT regimen); the other replaced both rifampin with RPT 1200mg and ethambutol with MOX 400mg (RPT-MOX regimen). The primary endpoints were TB disease-free survival 12 months after randomization for efficacy and grade 3 or higher severe adverse events (AEs) on treatment for safety. Randomization was stratified by cavitary disease and HIV-status; participants with HIV on efavirenz (EFV)-based ART were enrolled in a staged fashion, to allow studies of drug-drug interactions between RPT and EFV.

Results: We enrolled 2516 participants from 13 countries in sub-Saharan Africa, Asia, and the Americas; median age was 31 years, 71% were male, 64% were non-White, and 73% had cavitary disease. Two hundred fourteen (8%) had HIV infection; median age was 36 years, 59% were male, 99% were non-White, and 72% had cavitary disease. The median CD4+ count was 344 cells/mm3, and all were on EFV-based ART at baseline or started within 8 weeks of enrollment. Overall, the RPT-MOX regimen efficacy was non-inferior to control after 12 months of treatment for TB and all safety outcomes. The primary efficacy outcomes were similar across the two RPT-MOX arms, with slightly better outcomes in the arm that included ethambutol with MOX 400mg (RPT-MOX arm).

Conclusion: Daily rifapentine plus moxifloxacin is a non-inferior regimen for pulmonary tuberculosis in people with HIV. The regimen is well-tolerated and has the potential to shorten treatment duration.

HIGH-DOSE RIFAMPICIN FOR HIV-ASSOCIATED TB MENINGITIS: A PHASE II RANDOMISED TRIAL

Fiona Cresswell1, David Meyers2, Erick Kagimu3, Daniel Grint4, Lindsey te Brake5, John Kasibante6, Emily Martyn7, Morris Rutakbingira8, Lillian Tumuge2, Kenneth Ssebambulide1, Elin Svensson6, Rob Aarnoutse9, Anaanta Bangdiwala9, Alison Elliott2, David Boulware10, for Rift study
1London School of Hygiene & Tropical Medicine, London, UK, 2Infectious Disease Institute, Kampala, Uganda, 3Radboud University Medical Center, Nijmegen, Netherlands, 4University of Minnesota, Minneapolis, MN, USA

Background: Tuberculous meningitis (TBM) carries a high case-fatality of 50% in HIV co-infection. Treatment of TBM is challenging as rifampicin does not readily penetrate into cerebrospinal fluid (CSF) at standard dosing (10mg/kg). High dose rifampicin improves CSF rifampicin levels and survival in predominantly HIV-negative Asian adults with TB. However, intensified therapy for TBM has not been studied in African adults and there is a paucity of data on safety and pharmacokinetics (PK) of high dose rifampicin in HIV co-infection.

Methods: We conducted an open-label phase II trial (SRC/TN4218549) with three arms: A) intravenous rifampicin (20mg/kg/day, IV-20); B) oral rifampicin (35mg/kg/day, PO-35); C) standard of care (rifampicin 10mg/kg/day, SOC). Standard dosing of isoniazid, pyrazinamide, ethambutol were administered. Eligible adults in two Ugandan hospitals underwent PK sampling (0, 2, 4, 8, 12, 16, 18, 24 hours) on day two along with a CSF sample. Follow-up was 24 weeks. Standard non-comparative analysis and population PK modelling determined maximum concentration in serum (Cmax) and total exposure (AUC0-24).

Results: We randomised 61 adults, 56 (92%) were HIV-positive, median CD4=50 (IQR 46–56) cells/μl. M. tuberculosis in CSF was confirmed by Xpert MTB/RIF Ultra in 31 (51%). Compared to SOC, rifampicin IV-20 and PO-35 significantly increased geometric mean serum exposures: Cmax increased from 6.09mg/l to 36.2mg/l and 29.3mg/l (P<0.001), and AUC0-24 increased from 42.9mg-l to 248.7mg-h and 326.9mg-h (P<0.001) respectively. In CSF, with SOC, 10/18 (56%) had undetectable rifampicin (<0.25mg/l), geometric mean concentration was 0.27mg/l. CSF concentrations were increased to 1.74mg/l and 2.17mg/l in the IV-20 and PO-35 arms respectively (P<0.001). Rifampicin minimal inhibitory concentration (MIC) was reached in 11% of SOC, 93% of IV-20 and 95% of PO-35 adults. High dose rifampicin was safe, with no increase in grade 3-5 adverse events (P=0.74). Drug induced liver injury occurred in 1 (5%), 2 (10%) and 4 (19%) of participants in the IV-20, PO-35 and SOC arms respectively. Overall, 24 (39%) participants died, with no between-arm difference in mortality (P=0.48).

Conclusion: With standard TB treatment only 1 in 10 adults with TB had a CSF rifampicin concentration above the MIC. In a predominantly HIV-positive severely immunocompromised Ugandan population, high dose rifampicin was safe, resulted in significant increases in plasma and CSF exposures, and attainment of therapeutic CSF levels.
**132 BICTEGRAVIR CONCENTRATIONS AND Virologic RESPONSES IN PLWH RECEIVING 1HP FOR LTBI**

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1National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, 2National Taiwan University Hospital, Taipei, Taiwan, 3National Taiwan University College of Medicine, Taipei, Taiwan

**Background:** One-month regimen of daily rifapentine (600 mg) plus isoniazid (300 mg) (1HP) is an attractive preventive strategy for PLWH with latent TB infection (LTBI). However, the optimal integrase strand transfer inhibitor-based combination in combination with 1HP remain unknown. We aimed to assess the completion rate, bictegravir (BIC) trough concentration, cytokine profile, and virologic response in PLWH with LTBI who received 1HP and BIC/FTC/TAF concurrently.

**Methods:** PLWH were enrolled who tested positive by interferon gamma release assay (IGRA) and had received BIC/FTC/TAF for at least 2 weeks with plasma HIV RNA load (PVL) <200 copies/mL within 6 months of initiation of 1HP. BIC trough concentrations were determined with the use of LC-MS/MS before the first dose (Day 1), 24 hours after the 14th (Day 15) and 28th (Day 29) doses of 1HP, and PVLs were determined within 6 months before and 2 weeks after 1HP initiation (Day 15), end of 1HP (Day 29), and 3 months after 1HP discontinuation. Cytokine levels were determined with the use of commercial kits on Day 1, Day 15, and Day 29.

**Results:** From November 2019 to November 2020, a total of 1832 PLWH tested positive for LTBI with 59 (3.2%) being positive by IGRA. Fifty PLWH with LTBI who had received BIC/FTC/TAF for at least 2 weeks were enrolled for 1HP treatment (98% men and 90% MSM). Only one patient (2.0%) discontinued 1HP on Day 15 due to fever and generalized rashes; more than 5-fold increases of the IFN-gamma, TNF-alpha, and IL-10 levels were observed. Statistically insignificant increases of the cytokines assessed were observed in the remaining PLWH. Sequential changes of BIC trough concentrations after 1HP initiation were determined in 48 PLWH (Figure). The percentages of BIC trough concentration above the protein-adjusted 95% effective concentration (paeEC50=162 ng/mL) were 56.3% on Day 15 and 35.4% on Day 29. PVLs were <200 and <50 copies/mL in 100% and 97.9% of the enrolled PLWH before 1HP initiation (median 20 copies/mL and range 20-85 copies/mL, 97.9% and 72.9% on Day 15 (median 20 copies/mL and range 20-423 copies/mL), 97.9% and 91.5% on Day 29 (median 20 copies/mL, range 20-205 copies/mL), and 100% and 97.7% 3 months after 1HP discontinuation (median PVL 20 copies/mL, range 20-57 copies/mL).

**Conclusion:** BIC concentrations were significantly decreased with concurrent use of 1HP among PLWH with LTBI.

<table>
<thead>
<tr>
<th>Serum BIC C0 concentration (ng/mL)</th>
<th>IV-20</th>
<th>PO-15</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean (95% CI)</td>
<td>349</td>
<td>327</td>
<td>42.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.50</td>
<td>1.28</td>
<td>1.00</td>
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<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%) with concentration ≥ paeEC50</td>
<td>7 (3.8%)</td>
<td>6 (3.6%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum FTC C0 concentration (ng/mL)</th>
<th>IV-20</th>
<th>PO-15</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean (95% CI)</td>
<td>15.2</td>
<td>20.3</td>
<td>3.04</td>
<td>&lt;0.001</td>
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<tr>
<td>Ratio to control</td>
<td>1.31 (1.06-4.01)</td>
<td>1.66 (1.00-2.86)</td>
<td>0.177 (0.17-0.45)</td>
<td>0.058</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%) with concentration ≥ paeEC50</td>
<td>14 (93.9%)</td>
<td>18 (94.9%)</td>
<td>9 (64%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Day 1 (n=37)**

**Day 15 (n=48)**

**Day 29 (n=46)**

**4.5**

**4**

**3.5**

**3**

**2.5**

**2**

**1.5**

**1**

**0.5**

**0**

**37**

**133 A CLUSTER RANDOMIZED TRIAL OF CONTACT TRACING IN HOUSEHOLDS OF INDEX PATIENTS WITH TB**

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1Peninatal HIV Research Unit, Soweto, South Africa, 2London School of Hygiene & Tropical Medicine, London, UK, 3University of Limpopo, Polokwane, Limpopo, South Africa, 4Johns Hopkins Center for TB Research, Baltimore, MD, 5Liverpool School of Tropical Medicine, Liverpool, UK

**Background:** Household contact tracing and investigation for tuberculosis (TB) is recommended, but has not been widely implemented in high HIV-prevalence settings due to uncertainty over effectiveness and the costs of conducting home visits. Intensive HIV/TB screening with appropriate action could reduce household TB transmission and mortality.

**Methods:** Index TB patients in two health districts of South Africa with markedly different TB and HIV burdens (Mangaung in Free State and Capricorn in Limpopo) were randomised in a stratified manner, in a 1:1 ratio, to receive either an intensive household contact HIV/TB screening with referrals, or standard of care (SoC). In the intensive screening arm, consenting household members received: (1) collecting of sputum for TB testing (Xpert and culture) and point-of-care HIV testing for those without evidence of HIV-infection, with standardized treatment referral for those testing positive for TB and/or HIV, (2) home initiation of TB preventive therapy for HIV-infected individuals, children ≤5 years old, or tuberculosis skin test (TST) positive HIV seronegative individuals; and (3) a supportive household follow-up visit three months later. Index patients randomised to SoC received sufficient standardized referral letters for each of their household contacts without a household visit until the outcome visit. The primary outcome was TB-free survival, measured 15-months after randomization. Secondary outcomes were TST positivity in children <14 years and prevalence of undiagnosed or untreated HIV.

**Results:** Between December 2016 and March 2019, 1,032 households (4,129 contacts) and 1,030 (4,459 contacts) were randomised to the intervention and SoC arms. At 15-months, the percentage of household members who experienced incident TB or death did not differ between contacts from households that received intensive screening with referral (93/3,230, 2.9%) and those that receive SoC (80/2,600, 3.1%) (hazard ratio 0.90, 0.66-1.24). Prevalence of TST positivity was higher in the intensive screening arm (38/845, 4.5%) compared to the SoC arm (15/800, 1.9%, odds ratio: 2.25, 1.07-4.72). Prevalence of undiagnosed or untreated HIV was similar between arms (41/3185, 1.3% vs. 32/2543, 1.3%; OR 1.02, 0.64-1.64).

**Conclusion:** Despite being widely recommended, we were unable to demonstrate that a comprehensive household contact tracing strategy improved TB-free survival. Provision of contact referral letters to index patients with TB should be promoted over household visit strategies.
134 A CLUSTER RANDOMIZED TRIAL OF TARGETED UNIVERSAL TESTING FOR TB IN CLINICS
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1University of the Witwatersrand, Johannesburg, South Africa, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 3Boston University, Boston, MA, USA, 4Bill and Melinda Gates Foundation, Seattle, WA, USA, 5AQuity Innovations, Johannesburg, South Africa, 6TB/HIV Care Association, Cape Town, South Africa

Background: Many patients with tuberculosis (TB) disease are not diagnosed or started on TB treatment. Reliance on TB symptom screening misses patients with TB, especially HIV-infected individuals. We assessed if targeted universal testing for TB (TUTT) in adults at extreme risk of TB would increase the total number of patients diagnosed with TB in primary care clinics.

Methods: We conducted a cluster randomised trial, allocating 62 large clinics in three provinces in South Africa to either: augmentation of standard of care (SOC), symptom-based TB testing with the TUTT intervention, or to SOC. In TUTT clinics, we targeted high risk adult (≥18 years) clinic attendees, irrespective of the presence of TB symptoms. High risk individuals were: HIV-infected; close contacts of someone with TB in the past year; or prior TB in the past 2 years. Fieldworkers in TUTT clinics collected a sputum sample, which was processed and split for Xpert Ultra and mycobacterial culture. Outcome was the total number of TB patients diagnosed per clinic per month in each arm, measured using counts of laboratory diagnoses of TB at all clinics in the year prior, and during the intervention. We compared numbers of TB cases per month during the intervention between study arms, and also taking into account secular trends.

Results: From March 2019 to March 2020, we consented and sputum-tested 30,500 adults in TUTT intervention clinics. Most (71%) were HIV-infected. Overall 8% of all sputum tests were positive for M. tuberculosis on ≥1 assay. There was marked variation in effect of the intervention at individual clinics and by province but cluster- and province-adjusted comparison between TUTT and SOC clinics, restricted to the intervention period, showed a nonsignificant increase of 14% additional patients with TB diagnosed in TUTT clinics per month (95%CI: −6%; 38%). However, difference-in-differences analyses showed TB diagnoses per clinic per month in SOC clinics declined by 8% in the intervention period compared to the year prior, whereas TUTT clinics diagnosed 17% (95%CI: 14%; 19%) more patients relative to SOC.

Conclusion: Implementing targeted universal testing in high risk groups increased the number of TB patients by 17% compared to symptom-based TB testing, and strategies such as this may assist in eliminating TB.

135 A SIMPLE AND SAFE APPROACH TO HCV TREATMENT: FINDINGS FROM THE AS360 (MINMON) TRIAL
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1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2Instituto de Pesquisa Clinica Evandro Chagas, Rio de Janeiro, Brazil, 3Boston Harbor Chan School of Public Health, Boston, MA, USA, 4National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 5Social and Scientific Systems, a DHLCCompany, Silver Spring, MD, 6Massachusetts General Hospital, Boston, MA, USA, 7Gilead Sciences, Inc, Foster City, CA, USA, 8Thai Red Cross AIDS Research Center, Bangkok, Thailand, 9Cave Western Reserve University, Cleveland, OH, USA, 10Boston Medical Center, Boston, MA, USA, 11Duke University Medical Center, Durham, NC, USA, 12University of Colorado School of Medicine, Aurora, CO, USA

Background: To achieve global hepatitis C virus (HCV) elimination by 2030, 80% of the ~71 million people with chronic HCV need to be treated, necessitating simplification of treatment delivery and associated laboratory monitoring without compromising efficacy or safety. The COVID-19 pandemic has further highlighted the need for innovative models that minimize face-to-face contact.

Methods: ACTG AS360 is a single-arm, open-label trial to evaluate safety and efficacy of a minimal monitoring (MINMON) approach to HCV therapy in treatment-naive persons with no evidence of decompensated cirrhosis. All participants received a single-tablet, fixed-dose regimen of sofosbuvir/ribavatuvir for 12 weeks. MINMON included: (1) no genotyping; (2) all tablets dispensed at entry; (3) no on-treatment visits/labs; and (4) two remote contacts at Weeks 4 (adherence assessment) and 22 (scheduling sustained virology response [SVR] visit). Unplanned visits for participant concerns (related/unrelated to an adverse event [AE]) were allowed. SVR is defined as HCV RNA <lower limit of quantification at least 22 weeks after treatment initiation (missing HCV RNA = failure). 95% confidence intervals (CI) for SVR used Wilson's Score.

Results: 400 participants were enrolled from 10/2018–07/2019 at 38 sites in five countries across 4 continents; 399 initiated treatment. Median age was 47 years, 138 (35%) were cisgender women, 22 (6%) self-identified across the transgender spectrum, and 166 (42%) were White. At entry, 34 (9%) had compensated cirrhosis (FIB-4 ≥3.25) and 166 (42%) had HIV co-infection. Remote contact was successful at Weeks 4 and 22 for 394 (99%) and 335 (84%) participants, respectively. HCV RNA for SVR was available for 396 participants. Overall, 95% (379/399) achieved SVR (95% CI: 92.4%, 96.7%). SVR by country, biological sex, gender identity, age, cirrhosis status, HIV co-infection status and injection drug use are presented in Figure. Fifteen (3.8%) participants had unplanned visits; 3 were AE related and 6 were related to abnormalities during screening. Serious AE events through Week 24 visit were reported in 14 (3.5%) participants; none were treatment related or resulted in death.

Conclusion: In a diverse, global patient population, the MINMON approach to HCV treatment delivery was safe and achieved SVR comparable to current standards. Wider adoption of this approach coupled with innovative case-finding strategies may facilitate HCV elimination while minimizing in-person appointments and resource use.

136 HEPATOCELLULAR CARCINOMA AND HBV VIREMIA IN HIV/HBV-COINFECTED PERSONS IN NA-ACCORD
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1University of Washington, Seattle, WA, USA, 2University of Pennsylvania, Philadelphia, PA, USA, 3University of California San Diego, San Diego, CA, USA, 4University of Calgary, Calgary, Canada, 5University of British Columbia, Vancouver, Canada, 6Philadelphia FIGHT, Philadelphia, PA, USA, 7Yale University, New Haven, CT, USA, 8Kaiser Permanente, Oakland, CA, USA, 9Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 10University of California at San Diego, San Diego, CA, USA, 11McGill University, Montreal, Canada

Background: Chronic hepatitis B (HBV) is the predominant cause of hepatocellular carcinoma (HCC) worldwide. Although HBV coinfection is common in HIV, the determinants of HCC in HIV/HBV coinfection are poorly characterized. We examined the incidence and predictors of HCC in a multi-cohort study of HIV/HBV-coinfected individuals.

Methods: We examined all HIV/HBV-coinfected persons (defined by positive HBV surface antigen, e antigen, or HBV DNA) within 22 cohorts of the North American AIDS Cohort Collaboration on Research and Design from 1995–2016. The main outcome, first occurrence of HCC, was verified by medical record review and/or cancer registry. We used multivariable Cox regression to determine adjusted hazard ratios (aHRs [95% confidence intervals]) of factors assessed at cohort entry (age, sex, race, body mass index), ever during follow-up (hepatitis C coinfection, heavy alcohol use defined as alcohol use disorder diagnosis and/or ≥3 drinks/day or ≥7 drinks/week for females; ≥4 drinks/day or ≥7 drinks/week for males on AUDIT-C), or that were time-
updated (HIV RNA, CD4+ cell percentage, diabetes mellitus, HBV DNA, HBV-active antiviral treatment (ART)).

**Results:** Among 8,354 HIV/HBV-coinfected individuals (median age, 43 years; 93% male; 52.4% non-white), 115 HIV cases were diagnosed over 63,392 person-years of follow-up (incidence rate, 1.8 [95% CI, 1.5–2.1] events/1,000 person-years). Independent risk factors for HCC were: >40 years (aHR, 2.14 [1.36–3.37]), hepatitis C coinfection (aHR, 1.60 [1.07–2.39]), and heavy alcohol use (aHR, 1.51 [1.03–2.21]).

We time-updated HIV RNA >500 copies/mL (aHR, 0.88 [0.55–1.41]) and time-updated CD4+ percentage <14% (aHR, 1.03 [0.56–1.90]) were not. Among the 3,054 who had HBV DNA quantified, the risk of HCC was increased with HBV DNA >200 IU/mL (aHR, 2.70 [1.23–5.33]) and especially at >200,000 IU/mL (aHR, 4.34 [1.72–10.94]; see Table). HCC risk was also higher with each 1.0 log, IU/mL increase in HBV DNA (aHR, 1.18 [1.05–1.34]). HBV suppression <200 IU/mL with HBV-active ART for ≥1 year significantly reduced the risk of HCC (aHR, 0.42 [0.24–0.73]).

**Conclusion:** HBV/HIV-coinfected individuals at risk of developing HCC, with or without HBV suppression, may need to address other preventable risk factors.

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**137 UPRISING CIRCULATION OF HBV-COMPLEX PROFILES WITH HBsAg VACCINE-ESCAPE MUTATIONS**

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**Background:** Vaccine–escape mutations can alter HBsAg recognition by antibodies thus challenging vaccine efficacy, promoting immunosuppression-driven HBV-reactivation and impairing HBsAg detection by immunoassays. Limited information is available on the circulation of vaccine–escape mutations, single or in complex mutational profiles, over time and their impact on serological parameters in the setting of HBV genotype-D.

**Methods:** This study includes HBsAg sequences from 947 viremic HBV genotype-D infected patients (pts) collected from 2005 to 2019. 21 vaccine-escape mutations (T116N-P120E/S-T126A/I/N/S-Q129H/R-T131I/N-M133I/L-136V-V145I-A146V-I151V/W152R-Q165N) were identified. The first confirmed case of SARS-CoV-2 in North America was identified in Washington state (WA) on January 21, 2020. By October 1, 2020, there were 89,974 confirmed cases of SARS-CoV-2 in WA. To understand the role of epidemic seeding events, we estimated the number and timing of introductions of SARS-CoV-2 lineages in WA using phylogenetic methods.

**Methods:** Our analysis used full genome SARS-CoV-2 sequences from GISAID sampled between December 2019 and September 2020. In order to incorporate phylogenetic uncertainty into our estimates, we generated 25 samples of sequences each with 5 random polytomy resolutions. Each sample contained 4918 high quality WA sequences and 6504 non-WA sequences, including 5056 non-WA sequences that were closest to WA sequences based on the raw number of mutations and a time-stratified random sample of 1448 additional non-WA sequences. Sequences were aligned using MAFFT and phylogenetic trees were reconstructed by lineage (GISAID clades S, L/V, G/GH and GR) using IQTREE.

**Conclusion:** We estimated a median of 287 separate introductions (range 244–320) and 204 exported lineages (range 188–227) of SARS-CoV-2 into and out of WA state.

**Results:** We estimated a median of 287 separate introductions (range 244–320) and 204 exported lineages (range 188–227) of SARS-CoV-2 into and out of WA state. 204 exported lineages (range 188–227) of SARS-CoV-2 into and out of WA state.
139 FROM TESTING TO MORTALITY: COVID-19 AND THE INVERSE CARE LAW IN SWITZERLAND
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Background: The SARS-CoV-2 pandemic has created unprecedented challenges for society and healthcare systems worldwide. Switzerland is one of the more affected countries in Europe. We examined the association between socio-economic position (SEP) and SARS-CoV-2 tests, SARS-CoV-2-positive cases, COVID-19 hospitalisations and COVID-19 deaths in Switzerland.
Methods: We used surveillance data reported to the Federal Office of Public Health from March to October 2020. We geo-coded patients’ residential addresses to determine the Swiss neighbourhood index of SEP, based on education and occupation of household heads, rent per square meter, and crowding. We used negative binomial regression models adjusted for sex, age, canton of residence and wave of the epidemic (first, March to June; second, July to October) to investigate the association between deciles of the SEP index (1st=lowest, 10th=highest) and four outcomes. We used different denominators: the 2018 Swiss population for tests and deaths, the number of tests for positive cases, and the number of positive cases for hospitalisations.
Results: Analyses were based on 1,130,405 SARS-CoV-2 tests, 143,101 positive cases, 6,367 hospitalisations and 1,749 deaths up to 31 October 2020. Figure 1 shows the distribution across deciles of neighbourhood SEP of (A) tests per population, (B) positive cases per test, (C) hospitalisations per case and (D) deaths per population (the black lines and shaded areas show the corresponding model prediction adjusted for sex, age, canton of residence and wave of the epidemic – median posterior and 95% credible interval). The adjusted change in proportion per 1 decile increase in neighbourhood SEP was +2.4% (95% credible interval: 1.0 to 3.9) for tests per population, -2.4% (-3.6 to -1.1) for positive cases per test, -4.6% (-5.9 to -3.3) for hospitalisations per case and -4.5% (-7.7 to -1.4) for deaths per population.
Conclusion: This nation-wide study provides a comprehensive analysis of the association between SEP and SARS-CoV-2 testing, reported infections, and COVID-19-related hospitalisations and deaths. People living in neighbourhoods with higher SEP are more likely to be tested, but less likely to test positive, to be hospitalised or to die, a manifestation of the inverse care law where availability of care varies inversely with the need for it.

140 SEX/GENDER DIFFERENCES IN TESTING, PRESENTATION, AND OUTCOMES OF SARS-CoV-2 INFECTION
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Background: Males have increased rates of severe illness and mortality from SARS-CoV-2 compared to females. It is unknown whether this is due to differential care seeking, health status, illness presentation, comorbidities, and/or treatment responses. Understanding factors associated with sex/gender-based differences in COVID-19 outcomes is important for optimal care and therapeutics.

Methods: SARS-CoV-2 test positivity and admission rates were assessed between March and October of 2020 in the Johns Hopkins Medicine (JHM) system of five hospitals. Detailed patient-level data were extracted for hospitalized patients from the JH-CROWN, a COVID-19 registry utilizing the Hopkins Precision Medicine Analytics Platform. Descriptive statistics were used to analyze differences between males and females.
Results: 57% of 213,175 tests were done in females with a similar positivity rate (8.2% F vs 8.9% M). Males were more frequently hospitalized (28.4% F vs 33.4%). Of 2688 hospitalized, more males reported fever, whereas more females reported headache, loss of smell and vomiting (p<0.05). Females had more favorable presenting respiratory parameters with lower respiratory rates and higher SpO2/FiO2 ratios (p<0.001). There was a similar burden of comorbidities (Charlson score) but differences in specific comorbidities: obesity and asthma higher among females (p<0.001), heart disease (p=0.06), complicated hypertension (p<0.01), chronic kidney disease, smoking and alcohol use higher among males (p<0.001). Admission and peak lab values showed lower IL-6, ferritin, CRP, higher absolute lymphocyte count and lower neutrophil/lymphocyte ratio in females (p=0.001 for all), but no difference in D-dimer or ESR. Test of interaction between sex and age was significant for IL-6 and ferritin (I test, p<0.05). Males and females received medications against SARS-CoV-2 with similar frequency with exception of tocilizumab which was used more frequently in males. Males had a higher incidence of severe/death outcomes across all ages (28% vs 36%, p<0.001) and in particular among the 18–49 age group (11% vs 25%, p<0.001).
Conclusion: Females were less frequently admitted to the hospital after a diagnosis with SARS-CoV-2 infection. Despite an excess of obesity, females had a lower severity of respiratory parameters and lower inflammatory markers on presentation and had a lower frequency of severe outcomes from SARS-CoV-2 infection. Sex and age interactions with severe disease highlight critical risk features unique to males and females.

141 RACIAL DISPARITIES IN COVID-19 POSITIVITY AMONG PEOPLE LIVING WITH HIV IN THE US
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Background: Morbidity and mortality due to COVID-19 disproportionately impacts racial/ethnic minorities and adults with chronic diseases, potentially including people living with HIV (PLWH). Here, we present descriptive patient characteristics by COVID-19 positive and HIV status using the U.S. National COVID Cohort Collaborative (N3C).
Methods: Using N3C data, we conducted a retrospective cohort analysis of patients aged ≥ 18 years that had undergone COVID-19 testing. The N3C cohort includes patients with any encounter after 1/1/2020 with SARS-CoV-2 laboratory tests or diagnostic codes. Detailed electronic medical records are centrally gathered and data harmonized across health care organizations (34 sites). COVID-19 positivity was defined by a positive RT-PCR or antibody testing. HIV infection was defined based on standard diagnostic codes within 2 years prior to COVID-19 testing. Patient characteristics by COVID-19 positive and HIV status were compared using χ2 tests.
Results: Over 2.1 million patients were captured in the N3C as of 11/25/2020, of whom 292,226 (13.6%) were COVID-19 positive, 11,011 (0.5%) were PLWH of whom 1341 (12.2%) tested COVID-19 positive . Compared to HIV-negative patients with COVID-19, COVID-19-positive PLWH were more likely to be 45+ years of age (62.3% vs 43.8%, p<0.001), male (70% vs. 46%, p<0.001), treated on an outpatient basis (9% vs. 5%, p<0.001), and have a modified Charlson comorbidity index score of 3 or above (27% vs. 17%, p<0.001). COVID-19-positive PLWH were more likely to be NH-Black (51% vs. 45%, p<0.001) and Hispanic (8% vs. 5%, p<0.001), and, conversely, less likely to be NH-White (24% vs. 36%, p<0.001) when compared to PLWH without COVID-19 (Figure 1).
Conclusion: Racial/ethnic minorities, including NH-Black and Hispanic adults, are disproportionately affected by COVID-19 pandemic, including PLWH. Our ongoing analyses will shed light on underlying mechanisms, such as types of comorbidities, that may lead to racial/ethnic disparities in the concurrence of HIV and COVID-19 positivity in the US.

142 HIV AND COVID-19 INPATIENT OUTCOMES: A MATCHED RETROSPECTIVE MULTICENTRE ANALYSIS

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1Guy’s and St Thomas’ NHS Foundation Trust, London, UK, 2University College London, London, UK, 3Imperial College London, London, UK, 4The Pennine Acute Hospitals NHS Trust, Manchester, UK, 5St George’s University Hospital NHS Foundation Trust, London, UK, 6University Hospitals of Leicester NHS Trust, Leicester, UK, 7Imperial College Healthcare NHS Trust, London, UK, 8King’s College London, London, UK, 9Barts Health NHS Trust, London, UK

Background: Clinical outcomes for people living with HIV (PLWH) hospitalized with COVID-19 infections have shown mixed outcomes. We conducted a multicentre, UK retrospective matched cohorts’ analysis.

Methods: Index cases were HIV+ COVID-19 PCR+ patients hospitalized between dates 1st February - 31st May 2020. HIV-negative patients were matched to PLWH up to a 3:1 ratio across 6 sites in England, by hospital site, test date ±7 days, age ±5 years, gender, index of multiple deprivation decile (IMDD) ±1. The primary outcome was patients achieving ≥2-point improvement on a 7-point ordinal scale or discharge from hospital by day 28, whichever was earlier. Follow up was right-censored at day 28 for patients still in hospital. Baseline characteristics and outcomes were analysed by Cox-proportional hazards regression stratified by matching clusters using multiple imputation for missing data. The model adjusted for ethnicity, clinical frailty score, body mass index, baseline hypoxia, duration of symptoms, hypertension, diabetes, malignancy, cardiac, lung and renal disease.

Results: 68 PLWH and 181 HIV-negative patients were included. PLWH had an HR of 0.57 (95% CI 0.39, 0.85; p = 0.005) of achieving ≥2-point improvement or discharge compared to HIV-negative patients. The effect size of HIV-status was attenuated (aHR 0.70, 0.43, 1.17; P = 0.18) after adjustment in the multivariable model.握 baseline frailty (aHR = 0.79, 95% CI 0.65, 0.95; p = 0.011), malignancy (aHR = 0.37, 95% CI 0.17, 0.82; p = 0.04) having a greater impact on the primary outcome. Proportion of deaths (19.1% vs 19.3%, p = 0.266) and patients requiring ventilation (23.5% vs 17.1%, p = 0.25) were similar between PLWH and HIV-negative patients. Sensitivity analyses adjusting for age and imputation for missing data. The model adjusted for ethnicity, clinical frailty score, body mass index, baseline hypoxia, duration of symptoms, hypertension, diabetes, malignancy, cardiac, lung and renal disease.

143 CHANGES IN HIV TESTING SERVICES AFTER COVID-19 IN 11 SUB-SAHARAN AFRICAN COUNTRIES

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1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Centers for Disease Control and Prevention, Pretoria, South Africa, 3Centers for Disease Control and Prevention, Nairobi, Kenya, 4Centers for Disease Control and Prevention, Abuja, Nigeria, 5Centers for Disease Control and Prevention, Windhoek, Namibia, 6Government of Zambia Ministry of Health, Lusaka, Zambia, 7Centers for Disease Control and Prevention, Yaounde, Cameroon, 8Centers for Disease Control and Prevention, Abidjan, Cote d’Ivoire

Background: The COVID-19 pandemic has interrupted the implementation of many HIV prevention programs supported by the US President’s Emergency Plan for AIDS Relief (PEPFAR), especially in sub-Saharan Africa. We evaluated the effects of COVID-19 pandemic (e.g., lockdowns, lack of personal protective equipment, community fears) on efforts to reach the UNAIDS 90-90-90 targets by HIV case finding using index testing (IT) and provider-initiated testing and counseling (PTC) as well as HIV treatment initiation.

Methods: We conducted a descriptive analysis using programmatic data from persons aged 15 years and older reported to PEPFAR from 11 purposefully selected countries in sub-Saharan Africa. We calculated the percentage change in reported HIV case finding indicators during the COVID period, defined as January-June 2020, as compared to the pre-COVID period, during the same time frame in the preceding year, January-June 2019.

Results: Of the 11 countries, persons tested for HIV through PTC declined in seven (64%) and persons testing positive declined in 10 (91%), comparing the COVID to pre-COVID periods (see Table 1). Across all countries, total HIV testing and total number of persons testing positive by PTC decreased by 20% and 23% when comparing the COVID to the pre-COVID period, respectively. In parallel, five of the 11 countries (Cameron, DRC, Mozambique, Nigeria, South Africa) saw an increase in both IT and HIV case finding through IT, in COVID as compared to the pre-COVID period. Across all countries, total IT increased by 13% and HIV case finding through IT increased by 17% when comparing the COVID to the pre-COVID period. The number of HIV-positive people linked to treatment decreased in seven (64%) countries during the COVID period compared to pre-COVID. Across all countries, an increase of 3% in those HIV-positive people linked to treatment.

Conclusion: While testing through PTC decreased during the COVID period, testing and case finding through IT increased. The increase in IT may reflect the actions of healthcare facilities and providers to ensure that HIV-exposed individuals identified by an index case were still tested. Focusing on IT may help programs effectively identify HIV-positive people, especially during a pandemic or other disturbance.

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### Table 1: Changes in HIV Testing Services

<table>
<thead>
<tr>
<th>Country</th>
<th>Total IT Change</th>
<th>PTC Change</th>
<th>Positive Change</th>
<th>Treatment Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td>13% (10)</td>
<td>17% (9)</td>
<td>20% (10)</td>
<td>3% (7)</td>
</tr>
<tr>
<td>DRC</td>
<td>20% (11)</td>
<td>17% (10)</td>
<td>23% (11)</td>
<td>3% (6)</td>
</tr>
<tr>
<td>Mozambique</td>
<td>17% (9)</td>
<td>13% (7)</td>
<td>20% (9)</td>
<td>3% (5)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>23% (12)</td>
<td>17% (9)</td>
<td>20% (10)</td>
<td>3% (6)</td>
</tr>
<tr>
<td>South Africa</td>
<td>20% (11)</td>
<td>17% (10)</td>
<td>23% (11)</td>
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<tr>
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<td>20% (9)</td>
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<td>3% (6)</td>
</tr>
</tbody>
</table>

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**Note:** The table above shows the percentage change in total and PTC HIV testing and HIV positivity according to the comparison between the COVID and pre-COVID periods. The change in treatment initiation indicates the number of HIV-positive people linked to treatment in the respective periods.
Dramatic Decline in Public Sector HIV/STI Testing during SARS-CoV-2 Pandemic, Oregon

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Background: The SARS-CoV-2 pandemic has dramatically affected public health and STI programs in the US. We determined the impact of SARS-CoV-2 on public sector HIV and bacterial STI testing and diagnosis in Oregon.

Methods: We examined the periods of January through September of 2019 and 2020. We defined January through September 2019 and January through February 2020 as “before physical distancing,” March 2020 through May 2020 as “the height of physical distancing” and June through September 2020 as “ongoing physical distancing.” We used Poisson regression with robust standard errors to estimate incidence rate ratios and 95% confidence intervals (CI) comparing monthly HIV and bacterial STI testing and diagnosis rates during each phase of physical distancing.

Results: HIV and STI testing and diagnoses declined during physical distancing to nadirs in April and May 2020, with variable increases thereafter. Compared to before physical distancing, HIV, NG, CT, and syphilis testing decreased at both the height of (by 50%, 58%, and 59%, respectively) and during ongoing physical distancing (by 28%, 44%, and 38%, respectively). Testing did not return to 2019 levels by September 2020. Monthly CT cases and early non-primary non-secondary and late/unknown duration syphilis cases were lower during both phases of physical distancing compared to before physical distancing. NG cases decreased 23% (95% CI 2%–40%) at the height of physical distancing but were comparable before physical distancing and during ongoing physical distancing. Compared to before physical distancing, HIV cases were 36% lower at the height of physical distancing and 12% greater during ongoing physical distancing; neither difference was statistically significant. In contrast, primary and secondary syphilis cases were 45% (95% CI 22%–72%) greater during ongoing physical distancing.

Conclusion: Public sector HIV and bacterial STI testing declined following implementation of physical distancing measures for SARS-CoV-2 in Oregon. The large declines in testing likely reflect decreased access to utilization of screening services during physical distancing, as cases of CT and early non-primary non-secondary and late/unknown duration syphilis, infections that are likely to present asymptptomatically, decreased significantly. Primary and secondary syphilis diagnoses increased, however, indicating ongoing sexual risk during physical distancing. Lack of timely diagnosis and treatment of HIV/STI during physical distancing may lead to increased transmission.

145 COVID-19 Impact on Index Testing Services in 5 High HIV Prevalence Indian Districts

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Background: Routine HIV testing for partners and children of PLHIV (e.g., index testing) is a key component of HIV prevention. Ancillary information suggests that the COVID-19 pandemic’s lockdowns and subsequent economic and mobility restrictions have impacted HIV testing programs; however, there is limited empirical data demonstrating this.

Methods: Beginning in Oct 2019, we initiated index testing services in 5 high HIV prevalence districts in two Indian states (Maharashtra and Andhra Pradesh) at 55 sites (48 facility-based/7 community-based) to elicit and test contacts (spouses, sexual/needle-sharing partners, children) of known PLHIV. To assess the pandemic’s impact on index testing outcomes among contacts, we compared outcomes in a pre-pandemic period (Jan-Mar 2020) to two post-pandemic periods: 1) a lockdown period (Apr-June 2020), and 2) a post-lockdown period when restrictions were eased (July-Sept 2020). Specifically, we compared the index testing cascade: number of contacts tested, number of contacts testing HIV+, proportion testing HIV+, and proportion initiating ART, by period and setting (facility vs. community-based).

Results: In the pre-pandemic period, 3,191 contacts of 2,258 PLHIV were tested, among whom 859 tested HIV+ (27% positivity). By comparison, in the lockdown period, the number of contacts tested decreased by 84% (rate ratio [RR], 0.16; p<0.001) but positivity increased to 40%. Increases in the number tested were seen post-lockdown, but remained below pre-pandemic levels (RR, 0.54; p<0.001; Panel A). Overall, the pandemic’s impact was more severe in facility vs. community sites (Panel B). By Sept 2020, the number of contacts testing positive returned to near pre-pandemic levels in community sites but remained <50% in facility sites. The proportion of newly diagnosed contacts who initiated treatment increased from 81% HIV+ in the pre-pandemic to 88% in the lockdown and post-lockdown periods (p<0.01). The median time from diagnosis to ART initiation was 8 days pre-pandemic and during the lockdown, but reduced to 4 days post-lockdown.

Conclusion: The pandemic resulted in significant declines in the testing of contacts of PLHIV and new HIV diagnoses, however linkage to ART among those newly diagnosed remained high. Our findings suggest that expansion of community-based service sites and/or incorporating strategies such as HIV self-testing may be needed to regain and maintain progress towards UNAIDS 95–95–95 goals, given the ongoing impacts of COVID-19.
IMPACT OF VAGINAL STIs ON bNAb PROTECTION IN MACAQUES

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"Centers for Disease Control and Prevention, Atlanta, GA, USA; Beth Israel Deaconess Medical Center, Boston, MA, USA; Tomsk State University, Tomsk, Russia; University of Leuven, Leuven, Belgium; National Institutes of Health, Bethesda, MD, USA; Boston Children’s Hospital, Boston, MA, USA; National Institute of Allergy and Infectious Diseases, Rockville, MD, USA; and The Rockefeller University, New York, NY, USA"

Background: Broadly neutralizing antibodies (bNAbs) confer durable protection in macaques against repeated mucosal SHIV challenges and are being evaluated clinically for pre-exposure prophylaxis (PrEP). In a prior study, bNAb 10-1074 provided durable protection against repeated vaginal SHIV challenge in macaques where the plasma concentration at breakthrough was 0.1μg ml-1. Sexually transmitted infections (STIs) increase mucosal HIV infection risk and have the potential to reduce PrEP efficacy. To evaluate the impact of STIs on bNAb efficacy we have developed a novel macaque vaginal STI model combining Treponema pallidum (TP), Chlamydia trachomatis (CT) and Trichomonas vaginalis (TV) and used it to reassess 10-1074 mediated protection against vaginal SHIV challenges.

Methods: Six depot-medroxyprogesterone acetate (DMPA)-treated pigtail macaques were inoculated vaginally with TP (week -2, 4), CT (every 3 weeks), and TV (once weekly) from week -2 through end of study. Macaques were passively immunized with 10-1074 (100 μg kg-1) once via subcutaneous injection (week -1). Beginning at week 0, animals were challenged vaginally with low-dose SHIVSF162P3, once weekly until infection was confirmed via positive plasma viremia (LOQ= 60 vRNA copies per ml). Three DMPA-treated controls that were similarly inoculated with STIs, but received no antibody, were challenged repeatedly with SHIV until infection occurred. Among treated animals, longitudinal plasma samples were assayed via TZM-bl neutralization assay, using viruses pseudotyped with 10-1074-sensitive HIV Env (X2088.c9), to determine 10-1074 concentrations.

Results: STI-infected macaques that received 10-1074 were protected against a median of 12 SHIV challenges (range: 5-16), as compared to STI-infected untreated controls, which became infected following 2 challenges (P=0.0033, Log-rank test). Among macaques administered 10-1074, the median plasma bNAb concentration at the time of SHIV breakthrough was 1.0 μg ml-1 (range:0.6-9.9 μg ml-1).

Conclusion: A single subcutaneous administration of 10-1074 durably protected macaques against repeated vaginal SHIV challenges despite their being co-infected with ulcerative and nonulcerative vaginal STIs. However, higher bNAbs levels may be required for protection against vaginal SHIV infection in the presence of STIs. This finding may impact dose selection for clinical development and highlights the importance of additional preclinical testing of efficacy in STI models.

PHASE 1 PK, SAFETY, AND ACCEPTABILITY STUDY OF 3-MONTH DAPIVIRINE VAGINAL RINGS

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"Assistance Publique–Hôpitaux de Paris, Paris, France; Centre for Social Health, Paris, France; Centre for Social Health Checkpoint Paris, Paris, France; Institut National de la Santé et de la Recherche Médicale, Paris, France; Institut National de la Santé et de la Recherche Médicale, Marseille, France; Agence nationale pour le Sida et les hépatites virales, Paris, France; Coalition Plus, Paris, France; and The Johns Hopkins University School of Medicine, Baltimore, MD, USA"

Background: Vaginal rings (VRs) are a promising approach to long-acting HIV prevention. Extended duration VRs may reduce user burden and cost, streamline follow-up, and encourage adherence. We evaluated the safety, pharmacokinetics, adherence, and acceptability of two 3-month dapivirine (DPV) VRs compared with the monthly DPV VR.

Methods: MTHN-036/IPM-047 enrolled 49 HIV-negative participants into a Phase 1, multi-site, randomized (1:1:1) trial comparing two extended duration (100 or 200 mg DPV) VRs used continuously for 13 weeks to a monthly 25 mg DPV VR. DPV concentrations were quantified in plasma, cervicovaginal fluid (CVF), and cervical tissue, at nominal timepoints. Geometric means ratios (GMRs) relative to the comparator ring were estimated using a fixed-effects model on log-transformed outcomes. Used rings were analyzed for residual DPV levels. Safety was assessed by adverse events (AEs), acceptability and adherence by self-report.

Results: Mean age was 30.0 (range 19–44) years; 41% were Black, 39% white, 8% Asian, and 12% of other race. Retention was 94% through day 91. There were no differences in the proportion of participants with grade ≥2 genitourinary AEs or grade ≥3 AEs in the extended duration vs. monthly VR arms (P=1.0). Across timepoints, plasma and CVF DPV concentrations were higher in the 100 and 200 mg VR arms compared with the 25 mg arm (Table). Additionally, the peak concentration (C’), and Area Under the Concentration-Time Curve (AUC) for 0-28 days were 1.5 to 2 times higher for the extended duration VRs vs. monthly VR. Cervical tissue concentrations were consistently higher in the 200 mg VR (GMRs 2.36-3.97) and higher in the 100 mg VR at day 91 (GMR 3.04). A majority of participants (82%) reported being fully adherent, with no statistically significant differences between groups. Most participants reported liking the VRs (median IQR); 8 (6-10) on 10-point Likert scale and reported they were likely to use the VR if effective (median IQR); 9 (7-10) on a 10-point Likert scale of future VR use. Based on manufacturer-reported DPV loads, the mean total DPV released over 13 weeks was estimated to be 11.2 mg for the 25 mg VR, 14.2 mg for the 100 mg VR, and 22.8 mg for the 200 mg VR.

Conclusion: The extended duration DPV VRs were well-tolerated and achieved higher DPV concentrations compared with the monthly DPV VR, likely translating into at least equal efficacy. These findings support further evaluation of 3-month DPV VRs for HIV prevention in women.

TAD Table: Concentrations in Plasma (μg/ml), Cervicovaginal Fluid (μg/ml), and Cervical Tissue (μg/ml).

<table>
<thead>
<tr>
<th>TAD VR</th>
<th>Plasma Concentration</th>
<th>Cervicovaginal Fluid Concentration</th>
<th>Cervical Tissue Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg</td>
<td>2.4 (1.7-3.2)</td>
<td>1.2 (0.9-1.6)</td>
<td>0.3 (0.2-0.5)</td>
</tr>
<tr>
<td>100 mg</td>
<td>2.8 (2.1-3.6)</td>
<td>2.1 (1.7-2.7)</td>
<td>0.7 (0.5-1.1)</td>
</tr>
<tr>
<td>200 mg</td>
<td>2.4 (1.9-3.0)</td>
<td>1.9 (1.5-2.4)</td>
<td>0.6 (0.4-0.9)</td>
</tr>
</tbody>
</table>

INCIDENCE OF HIV INFECTION WITH DAILY OR ON-DEMAND ORAL PRep WITH TDF/FTC IN FRANCE

Jean-Michel Molina, Jade Ghosh, Constance Delaugerre, Gilles Plaixou, Christine Katlama, Laurence Slama, Claire Pintado, Michel Ohayon, Hannane Mouhimi, Lambert Assoumou, Bruno Spire, Mohamed Ben Mechania, Daniela Rojas Castro, Dominique Costagliola, for ANRS Prevenir study group

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Background: On-demand PrEP with TDF/FTC has been recommended as an alternative to daily PrEP for MSM by EACS, WHO and IAS-USA guidelines, but has not been endorsed yet by CDC due to limited real-world experience.

Methods: The ANRS Prevenir study is an ongoing prospective cohort study enrolling individuals at high risk for HIV infection on PrEP. MSM could opt for either daily or on-demand PrEP with TDF/FTC. At baseline, month 1 and every 3 months thereafter, subjects were tested for HIV using a 4th generation combined ELISA assay and other STIs and creatinine plasma levels were monitored. At each visit participants provided information regarding sexual behaviour, dosing regimen and drug adherence. Our main objective was to assess the overall HIV incidence in the study and per dosing regimen, as well as incidence of bacterial STIs (including syphilis, gonorrhoea, chlamydia and Mycoplasma genitalium) and viral hepatitis. Safety and study retention were also assessed. This analysis uses data accumulated up to September 30, 2020.

Results: From May 3rd 2017 to March 2nd 2019, 3067 subjects were enrolled across 22 sites in the Paris region, 44% being PrEP naive. Median age was 36 years (IQR: 29-43), 98.5% were MSM. At enrolment, PrEP was used daily and on demand by 50.5% and 49.5% of participants, respectively. Median number of partners in the last 3 months was 10 (5-20) and median number of condomless sex events in the prior 4 weeks was 2 (0-5). Median follow-up lasted 22 months and accumulated 5633 person-years (PY) with an overall HIV incidence in the cohort of 0.11% (95% CI: 0.04-0.23) per 100 PY. Six participants (3 daily, 3 on demand) acquired HIV-infection during the study period (P=0.99). Condom use at last sexual intercourse was 19.6%. Overall STIs incidence was 73 (95% CI:
70.7–75.5) per 100 PY which remained stable during follow-up except during the COVID-19 lockdown when it dropped to 32.4 per 100 PY (P<0.01). HCV incidence was 0.69 per 100 PY. Incidence of participants lost to follow-up was 10.3/100 PY and 19 subjects (0.6%) discontinued PrEP for drug-related adverse events (gastrointestinal: 12, e-GFR < 70 ml/min: 4, other: 3).

Conclusion: In this PrEP cohort, enrolling mainly MSM at high risk of HIV-acquisition in Paris, HIV incidence was low whether participants used daily or on demand PrEP. There was a high incidence of bacterial STIs and HCV infection despite a drop in STIs incidence during the COVID-19 lockdown.

149 IMPACT OF COMMON SIDE EFFECTS ON PrEP PERSISTENCE DURING PREGNANCY IN SOUTH AFRICA
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1University of Cape Town, Cape Town, South Africa, 2Desmond Tutu HIV Foundation, Cape Town, South Africa, 3University of California Los Angeles, Los Angeles, CA, USA

Background: Oral pre-exposure prophylaxis (PrEP) is a safe and effective prevention strategy to reduce women’s risk of HIV in pregnancy and postpartum. Effective PrEP requires daily PrEP adherence, but little is known about how minor symptoms, which may be more common during pregnancy, overlap with PrEP side effects (SE) and could impact on PrEP persistence.

Methods: The PrEP in pregnancy and postpartum (PrEP-PP) study is an ongoing prospective cohort that enrolls consenting pregnant, HIV-uninfected women (>15-years) at first antenatal care (ANC) visit, followed through 12-months postpartum. Interviewers collected data on socio-demographics, SE, and PrEP use. We analyzed the reporting of SE and their association with PrEP persistence (defined as staying on PrEP at 3-months) and PrEP adherence (defined as taking PrEP >5 of last 7 days at 3-month visit) with multivariable logistic regression adjusting for baseline maternal, gestational age, and time in study.

Results: Between August 2019 and November 2020 we enrolled 759 pregnant women (median gestation=21 weeks; median age=26 years). Following PrEP counseling, 91% of pregnant women initiated PrEP at their first antenatal visit (n=690), including 21 women <18 years old (84%); 20% were married. Overall 73% of women on PrEP returned for a repeat prescription at 1-month, and 62% returned at 3-months. Among those returning at 3-months, 85% reported adhering to PrEP. Adherence was poorer with women who came in later in their pregnancy (>20 weeks) for their first ANC visit, or had lower education (completed primary vs. secondary school) (p<0.05). Over 31% of women on PrEP reported side effects at 1-month, mostly nausea/vomiting (22%), dizziness (25%), and headache (8%). Women on PrEP in the 1st or 2nd trimester had highest odds of reporting side effects (aOR=2.61; 95%CI=1.17, 5.84) compared to postpartum women adjusting for age, gestation and time in study (Table 1).

Conclusion: PrEP initiation was high in antenatal care in this setting but reporting of side effects that may be overlap with pregnancy symptoms was associated with poor PrEP persistence and adherence. This presents an opportunity for improved clinical management and counseling during pregnancy of nausea/vomiting to normalize early, transient side effects to improve PrEP adherence in pregnant women.

Table 1. Factors associated with poor PrEP persistence & adherence at 3-months after initiation among pregnant women in Cape Town

<table>
<thead>
<tr>
<th>Gestational age &gt;20 weeks at first ANC visit</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted odds ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenesis (primary vs. secondary or higher)</td>
<td>0.60 (0.35, 0.93)</td>
<td>0.55 (0.32, 0.93)</td>
</tr>
<tr>
<td>Reported side effects on PrEP</td>
<td>0.56 (0.39, 0.81)</td>
<td>0.50 (0.31, 0.81)</td>
</tr>
</tbody>
</table>

*Adjusted for age, gestational age and follow-up time

150 COST-EFFECTIVENESS OF LONG-ACTING PrEP AMONG MSM/TGW IN THE US
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Background: HIV Prevention Trials Network (HPTN) 083 demonstrated superior efficacy of long-acting injectable cabotegravir (CAB-LA) compared to oral tenofovir disoproxil fumarate/efavirenz (FTD) for HIV pre-exposure prophylaxis (PrEP). CAB-LA cost may be higher than that of generic F/TDF. We projected the clinical benefit of CAB-LA vs. F/TDF and estimated the cost at which CAB-LA would be cost-effective.

Methods: Using the Cost-Effectiveness of Preventing AIDS Complications (CEPAC) simulation model, we examined 2 strategies: generic F/TDF (or branded F/TAF) and CAB-LA among high-risk men who have sex with men and transgender women (MSM/TGW, i.e. trial-eligible) starting PrEP in the US (n=476,300). We used trial and published data including: HIV incidence (off PrEP: 5.32/100PY; F/TDF (and F/TAF): 1.33/100PY; CAB-LA: 0.26/100PY); HIV transmissions off-PrEP attributable to high-risk MSM/TGW: 7,800/year (yr); 62% 6yr-PrEP retention. We assumed constant incidence and annual transmissions. Annual costs were: generic F/TDF 88,400 ($16,900); CAB-LA 28,000, and ART: 24,500 ($39,600). Projected outcomes included HIV transmissions averted, quality-adjusted life-years (QALYs), costs, and incremental cost-effectiveness ratios (ICERS, $/QALY) over 10 yrs. We used a willingness-to-pay threshold of $100,000/QALY. In sensitivity analysis, we varied PrEP costs and transmissions/yr. We also examined providing CAB-LA to all PrEP-eligible MSM/TGW (n=1,905,300) — not just those at high risk — with HIV incidence off PrEP: 1.54/100PY and HIV transmissions: 19,700/yr.

Results: In the base case, compared to generic F/TDF (or branded F/TAF), CAB-LA increased life expectancy by 37,000 QALYs (37,000 QALYs) and costs by $36,789 ($20,148), leading to an ICER of $994,000/QALY ($544,000/QALY, Table). CAB-LA would be cost-effective compared to F/TDF or F/TAF over 10yrs at a maximum price premium over F/TDF (F/TAF) of $700/yr ($1,800/yr). When offered to all PrEP-eligible MSM/TGW, CAB-LA would be cost-effective over 10yrs at a maximum price premium of $200/yr (vs. F/T DF or F/T AF) and $500/yr (vs. F/T AF).

Conclusion: The superiority of long-acting injectable PrEP notwithstanding, the presence of highly effective alternatives limits the additional price difference that payers should be willing to pay for CAB-LA.

Table. Model-projected clinical, cost, and cost-effectiveness outcomes of long-acting injectable cabotegravir (CAB-LA) for HIV pre-exposure prophylaxis (PrEP) compared to generic F/TDF over a 10-year horizon

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Transmissions</th>
<th>Incremental QALY</th>
<th>Incremental cost, billion USD</th>
<th>ICER, $/QALY</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk MSM</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>F/TDF</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CAB-LA</td>
<td>8,000</td>
<td>37,000</td>
<td>26.78</td>
<td>944,000</td>
</tr>
<tr>
<td>All prevention eligible MSM</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>F/TDF</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CAB-LA</td>
<td>9,000</td>
<td>40,000</td>
<td>140.87</td>
<td>3,322,000</td>
</tr>
</tbody>
</table>

| High risk MSM | -- | -- | -- | -- |
| F/TDF | -- | -- | -- | -- |
| CAB-LA | 8,000 | 37,000 | 20.14 | 544,000 |
| All prevention eligible MSM | -- | -- | -- | -- |
| F/TDF | -- | -- | -- | -- |
| CAB-LA | 5,000 | 40,000 | 78.18 | 1,800,000 |


Results are discounted at 3 percent per year and rounded to the nearest thousand. The ICER is the difference in cost divided by the difference in life expectancy for each strategy compared with the next least costly strategy.

151 SOCIAL NETWORKS PREDICT PrEP UPTAKE IN STUDY IN RESEARCH IN RURAL KENYA AND UGANDA

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1University of California San Francisco, San Francisco, CA, USA, 2University of Massachusetts Amherst, Amherst, MA, USA, 3Kenya Medical Research Institute, Nairobi, Kenya, 4Infectious Diseases Research Collaboration, Kampala, Uganda, 5University of California Berkeley, Berkeley, CA, USA, 6Makerere University College of Health Sciences, Kampala, Uganda

Background: Peer support may be important for increasing PrEP use. However, little is known about the influence of social networks (connections between

Methods: The SEARCH study (NCT01864603) offered TDF/FTC PrEP in 16 communities during population-level HIV testing starting in 2016-2017. Universal PrEP access with rapid or same-day start was offered to HIV-uninfected adults ≥15 years, with enhanced PrEP counseling for those at elevated HIV risk (based on serodifferently partnered risk, risk score, or self-identified risk). During population-level testing, persons were asked to name social contacts in 5 domains: health, money, emotional support, food, and free time. Named contacts were matched to community residents to build community-specific, sociocentric networks of 56,770 persons and 124,054 connections. Using targeted maximum likelihood estimation, we evaluated social network predictors of PrEP uptake within 1 year of population-level testing among persons at elevated HIV risk who had ≥1 matched first-degree contact, accounting for clustering by community and adjusting for sociodemographic factors (sex, age, serodifferent partner, polygamous marriage, mobility, occupation).

Results: Among 13,159 persons at elevated HIV risk, 8,898 (68%) had ≥1 matched network contact. Of the 8,898, 49% were women, 34% ages 15-24 years, 11% had a serodifferent partner, 14% had ≥1 contact who started PrEP, and 18% had ≥1 contact living with HIV (LHIV). Overall, 2,570/8,898 (29%) started PrEP. Persons with ≥1 contact who started PrEP were 57% more likely to start PrEP (adjusted risk ratio [aRR] 1.57, 95% CI 1.44-1.70, p<0.001) than those with contacts who did not start PrEP (Figure). Results were similar when stratified by sex and for same-sex and opposite-sex social contacts. Having an opposite-sex contact LHIV was associated with PrEP uptake in unadjusted analyses (RR 1.30, 95%CI 1.05-1.61, p=0.009), but not after adjustment for serodifferent partners and other factors (aRR 0.93, 95%CI 0.77-1.12, p=0.39).

Conclusion: Persons with a social contact who initiated PrEP were more likely to themselves start PrEP within 1 year of PrEP offer during population-level HIV testing. Interventions to facilitate peer support and strengthen social connections to other PrEP users should be considered to foster PrEP uptake.

152 HSV-2 ACQUISITION IN A RANDOMIZED TRIAL OF CONTRACEPTIVE METHODS AMONG AFRICAN WOMEN

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Background: Herpes simplex type 2 (HSV-2) infection causes recurrent genital ulcer disease and perinatal morbidity and is a strong risk factor for HIV acquisition. Women have higher HSV-2 prevalence than men, and conflicting data from observational studies suggest a possible association of HSV-2 acquisition with use of certain contraceptive methods, particularly intramuscular depot medroxyprogesterone acetate (DMPA-IM).

Methods: Within a randomized trial of the effect of three contraceptive methods – DMPA-IM, a copper intrauterine device (IUD), and a levonorgestrel (LNG) Iimplant – on HIV acquisition, we assessed HSV-2 acquisition among women HSV-2 seronegative at baseline. Women who were HIV seronegative, aged 16-35 years, and seeking effective contraception were recruited from 12 sites in Kenya, the Kingdom of Eswatini, South Africa, and Zambia from 2013-2017. Follow-up was quarterly for 12-18 months, with HSV-2 serologic and confirmatory Western Blot testing done at enrollment and the final study visit according to a predetermined algorithm. Intention to treat analysis using Poisson regression with robust standard errors was done adjusting for age less than 25 years, having living children, living with a husband or primary partner, vaginal sex without a condom, having more than one sex partner, and having a new sex partner.

Results: Amongst 7829 HIV negative women randomized, 4062 (52%) were HSV-2 seronegative at baseline and 3898 had a conclusive HSV-2 result at the final visit. Of these, 614 (16%) acquired HSV-2 at an incidence of 12.4/100 person-years (p-y). HSV-2 incidence was 10.9/100 p-y among women assigned DMPA-IM, 13.7/100 p-y among those assigned the copper IUD, and 12.7/100 p-y among those assigned the LNG implant. Incidence rate ratios (IRR) for HSV-2 acquisition were 0.81 (95% confidence interval [CI] 0.67-0.99, p=0.04) for DMPA-IM compared with copper IUD, 0.86 (95%CI 0.71-1.05, p=0.15) for DMPA-IM compared with LNG implant, and 0.94 (95% CI: 0.78-1.13, p=0.50) for LNG implant compared with copper IUD. HSV-2 acquisition was associated with HIV seroversion (IRR 3.6, 95% CI 2.9-4.6) during follow-up and detection of Neisseria gonorrhoeae (IRR 1.9, 95% CI 1.4-2.5) and Chlamydia trachomatis (IRR 1.4, 95% CI 1.2-1.7) infection at the final study visit.

Conclusion: HSV-2 incidence was high among this population of young African women and was not strongly associated with contraceptive method. HSV-2 incidence was lowest for DMPA-IM users.

LABORATORY ANALYSIS OF HIV INFECTIONS IN HPTN 083: INJECTABLE CAB FOR PrEP

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Background: HPTN 083 showed a 66% reduction in HIV incidence in cisgender men and transgender women who have sex with men (MSM/TGW) randomized to cabotegravir (CAB) 600 mg injections every 8 weeks (after an oral lead-in) vs. daily tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for pre-exposure prophylaxis (PrEP). We originally reported 52 incident infections among 4566 participants (13 CAB, 39 TDF/FTC; annual incidence: 0.41% vs. 0.51%). Here we assess the 58 cases to better characterize the 58 cases (Table).

Methods: Concentrations of CAB and tenofovir (TFV) in plasma and TFV-diphosphate in dried blood spots were quantified by liquid chromatography-tandem mass spectrometry. HIV status and timing of HIV infection were assessed with an antigen/antibody (Ag/Ab) test, a discriminatory test, and RNA assays. Drug resistance testing was performed for samples with HIV RNA >500 copies/mL.

Results: Among 12 incident infections in the CAB arm: 5 had no recent CAB dosing; 3 occurred in the oral lead-in phase (1 had no CAB detected); 4 occurred despite on-time CAB injections and targeted CAB concentrations. Five of the 16 infections in the CAB arm had integrase resistance associated mutations (RAMs; Q148R or Q148K with accessory mutations, or R263K); 1 of these cases also had a non-nucleoside reverse transcriptase inhibitor (NNRTI) RAM. One case had NNRTI RAMs only and 1 had NNRTI RAMs with nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) RAMs. In the TDF/FTC arm, 37/93 incident
infections occurred in participants with drug concentrations indicating suboptimal or non-adherence. One infection was likely due to transmission of TDF/FTC-resistant HIV; 1 occurred despite targeted drug concentrations. Thirteen of the 42 infections in the TDF/FTC arm had RAMs: 3 had NRTI RAMs only; 3 had NNRTI and NRTI RAMs; and 2 had NNRTI RAMs only. Retrospective HIV RNA testing identified HIV infection earlier than Ag/Ab testing performed at study sites.

**Conclusions:** TDF/FTC and CAB are highly effective for HIV PrEP in MSM/TGW. Oral pill non-adherence likely contributed to higher HIV incidence among those randomized to TDF/FTC. Integrase inhibitor resistance was observed in some cases in the CAB arm. Long-acting CAB is an important addition to HIV prevention options.

### Study Arm

<table>
<thead>
<tr>
<th>Study Arm</th>
<th>CAB Arm</th>
<th>TDF/FTC Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident infections</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Baseline infections</td>
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<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>42</td>
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</tbody>
</table>

154 **INFECTED CELLS ARE CLONOTYPICALLY DIVERSE IN BLOOD & LYMPH NODES SINCE FIEBIG STAGE I**

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**Background:** The initial cellular targets of HIV in lymphoid tissues remain unclear. Here, we used a single-cell approach to retrieve the phenotype, HIV envelope and T-cell receptor (TCR) sequences of HIV-infected cells in blood and lymph nodes from individuals at the earliest stage of HIV infection, prior to ART initiation.

**Methods:** Cross-sectional paired blood and lymph nodes samples from 21 acutely infected participants (n=5–3 individuals/Fiebig stage I to VI) and 4 untreated chronically-infected controls enrolled in the RV254/RV304 studies (Bangkok, Thailand) were analyzed. The phenotype of productively infected (p24+) cells was analyzed by multiparameter flow-cytometry (HIV-Flow). Clonotype and viral characterization of single sorted infected cells was determined by TCRβ and Env (C2V5) sequencing, respectively.

**Results:** Productively infected p24+ cells were detected in blood and lymph nodes since the earliest stage of acute infection (Fiebig I) and their frequency increased with time. Phenotyping analysis of 12,067 p24+ cells from blood and lymph nodes showed that memory cells expressing CCR5, HIV, CXCR4 and CXCR3 were preferentially infected at all stages. UMAP analysis revealed that infected cells were distributed in 11 cell clusters, and that their relative contribution to the overall pool of infected cells evolved rapidly across the different stages of acute infection and differed between blood and lymph nodes (Fig. 1). Although T follicular helper (Tfh) cells and particularly germinal center-Tfh were preferential HIV targets in lymph nodes during chronic infection, their contributions to the initial pool of infected cells were modest in acute infection. HIV-Env sequencing of 582 single-sorted p24+ cells showed a homogeneous population of HIV variants across blood and lymph nodes during all stages of acute infection, which diversified during chronic infection. In sharp contrast, distinct TCRβ were found in >99% of the cellular HIV targets (n=1,006 p24+ cells), indicating that initially infected cells were the product of independent infection events and that expansions in the pool of productively infected cells were rare early in infection.

**Conclusion:** HIV infection is established by a limited number of HIV variants infecting a large pool of phenotypically and clonotypically distinct memory T cells in both blood and lymph nodes. The phenotype of infected cells differed between Fiebig stages, suggesting a rapid temporal evolution in HIV cellular targets during acute HIV infection.

155 **EVOLUTIONARY DYNAMICS OF HIV RESERVOIR CELLS VIA A NOVEL SINGLE-CELL MULTITHEPNI ASSAY**

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**Background:** Latent reservoirs constitute a major barrier to HIV cure. Viral reservoir cell persistence may depend on proviral sequence, integration site and HIV transcription, but technical limitations have hindered efforts to obtain all three features from single infected cells. Here, we describe a novel technology that accomplishes this.

**Methods:** PBMC from 3 HIV+ patients, collected during pre-ART viremia and over 9+ years of continuous ART, were analyzed by a novel assay termed Parallel HIV RNA, Integration Site and Proviral Sequencing (PRIP-Seq). PBMC were diluted to single viral reservoir cells and subjected to parallel extraction of cellular DNA and RNA. Near-full-length proviral genomes and integration sites were obtained from DNA samples, and viral transcripts were detected by RT-ddPCR in corresponding RNA samples. Integration sites were annotated with genome-wide RNA-Seq, ATAC-Seq, ChiP-Seq, Hi-C and bisulfit sequencing data.

**Results:** Paired HIV RNA expression profiles and proviral sequences were determined for 872 individual proviruses. HIV RNA was detected in 45% and 35% of cells harboring genome-intact and defective proviruses, respectively. Integration sites were simultaneously obtained for 468 of these cells. Across all patients and timepoints, proviruses in non-genic regions were less likely to express HIV RNA when compared to genic integrants (18% vs. 37%, p=0.003), and these non-genic regions were associated with multiple epigenetic features of repressive chromatin. Proviruses in satellite DNA produced no detectable RNA. Among proviruses in genomes, HIV transcriptional silence correlated with increased upstream DNA methylation and reduced ATAC-Seq/activating CHIP-Seq reads in proviral 3D chromosomal contact regions. Longitudinal analysis suggested progressive enrichment of proviruses with reduced transcription and genomic/epigenetic integration site features of deeper latency during long-term ART, particularly among intact proviruses. However, several large proviral clones persisted for years despite ongoing HIV gene expression; these clones were located in close proximity to strong activating epigenetic chromatin marks.

**Conclusion:** Even after 9+ years of ART, a remarkable proportion of proviruses can be transcriptionally active, but these cells appear more vulnerable to immune-mediated clearance. Conversely, silent proviruses may have a survival advantage during long-term ART, seemingly due to distinct genomic/epigenetic features at integration sites and their 3D contact regions.

156 **NONINVASIVE PLASMA GLYCOMIC AND METABOLIC BIOMARKERS OF POSTTREATMENT HIV CONTROL**

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**Background:** Non-invasive biomarkers that predict HIV remission after antiretroviral therapy (ART) interruption are urgently needed. Such biomarkers can improve the safety of analytic treatment interruption (ATI) and provide mechanistic insights into the pathways involved in post-ART HIV control.
Methods: We examined two ATI cohorts: 1) Philadelphia cohort: a cohort of 24 HIV+ ART-suppressed individuals; 2) ACTG cohort: 74 participants of six ATI studies, including 47 non-controllers (NCs) and 27 post-treatment controllers (PTCs), a rare population sustains virologic suppression after ART-cessation. Pre-ART plasma metabolome was measured by mass spectrometry. Pre-ART plasma and IgG glycomes were profiled using capillary electrophoresis and lectin microarray. The J-Lat and THP-1 cell lines were used to examine latent HIV reactivation and myeloid inflammation, respectively. Cox and Logistic regression models with or without adjusting for confounders (age, gender, ethnicity, ART initiation, ART duration, and pre-ART CD4 count) were used for statistical analyses. False discovery rate (FDR) was calculated to account for multiple comparisons.

Results: We identified 13 plasma glycans and metabolites that their pre-ART levels associated with either faster (n=5; mostly pro-inflammatory molecules) or delayed (n=8; mostly anti-inflammatory molecules) viral rebound post-ART in both discovery and validation cohorts (hazard ratios ≥2 or ≤0.5; FDR<10%; P<0.05 after adjusting for confounders mentioned above). The pre-ART levels of 10 of these markers were significantly different between PTCs and NC (FDR<0.05). Among the top markers, pre-ART levels of L-glutamic acid predicted delayed rebound (P=0.005) and were higher in PTCs compared to NCs (P=0.0096). In vitro, L-glutamic acid significantly inhibited PMA or oC3/IC3D28 mediated latent HIV transcription in the J-Lat HIV latency model (P<0.0001). L-glutamic acid also prevented THP-1 inflammation upon LPS stimulation (P<0.02). Finally, using the machine learning algorithm, Lasso (least absolute shrinkage and selection operator), we combined this set of biomarkers into two multivariate models: Cox model that predicts time-to-viral-rebound with 74-76% capacity; and logistic model that predicts probability-of-viral-rebound (PVR score) with 97.5% capacity.

Conclusion: We fill a major gap in HIV cure research by identifying non-invasive biomarkers, with potential functional significance, that predict duration and probability of viral remission after treatment interruption.

PD-1 BLOCKADE ENHANCES THERAPEUTIC BENEFITS OF VACCINE IN A CHRONIC SIV/MACAQUE MODEL


Methods: We examined two ATI cohorts: 1) Philadelphia cohort: a cohort of 24 HIV+ ART-suppressed individuals; 2) ACTG cohort: 74 participants of six ATI studies, including 47 non-controllers (NCs) and 27 post-treatment controllers (PTCs), a rare population sustains virologic suppression after ART-cessation. Pre-ART plasma metabolome was measured by mass spectrometry. Pre-ART plasma and IgG glycomes were profiled using capillary electrophoresis and lectin microarray. The J-Lat and THP-1 cell lines were used to examine latent HIV reactivation and myeloid inflammation, respectively. Cox and Logistic regression models with or without adjusting for confounders (age, gender, ethnicity, ART initiation, ART duration, and pre-ART CD4 count) were used for statistical analyses. False discovery rate (FDR) was calculated to account for multiple comparisons.

Results: We showed that CD40L adjuvated DNA/MVA vaccine induced highly functional SIV-specific CD4+ and CD8+ T cell responses in blood, gut, and lymph nodes (LN) under anti-retroviral therapy. Combining PD-1 blockade with vaccine markedly increased the frequency of granzyme B+ perforin+ CD8+ T cells in blood and LN, enhanced their localization to BCF, and reduced viral reservoir. Upon ART interruption, combination therapy showed marked preservation of the granzyme B+ CD8+ T cells in the T cell zone and BCF regions of LN, maintained high SIV antigen-recognition breadth, showed notable control of reemerging viremia, and significantly improved survival, but not vaccine alone or control animals.

Conclusion: Our findings reveal that PD-1 blockade enhances the therapeutic benefits of vaccination by improving and sustaining the function and localization of vaccine-induced CD8 T cells to BCF and decreasing viral reservoirs.

TYPE I INTERFERON-ASSOCIATED GENE EXPRESSION PREDICTS TIME TO VIRAL REBOUND ON TI

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Methods: To explore the molecular differences between early and late rebounders, we present a longitudinal analysis of the host transcriptome that explores key genetic signatures associated with time to HIV rebound. We sequenced expressed host mRNA from South African women enrolled in the SPARTAC clinical trial, and who received treatment for up to 48 weeks, starting in primary infection. We studied CD4+ T-cells sampled at treatment interruption (wk 48). We used DESeq2 to quantify and transform our data, Gene Set Enrichment Analysis (GSEA) with the Reactome database and Weighted Gene Correlation Network Analysis (WGCNA) to identify putative genetic signatures associated with clinical outcomes. Genes identified by WGCNA to be correlated with time to rebound were screened using Cox Regression with multiple comparisons. False discovery rate (FDR) was calculated to account for multiple comparisons.

Results: GSEA showed an IFN-I response pathway enrichment in sustained controllers (SC) (>500 days to rebound) versus non-sustained controllers (non-SC) (<500 days to rebound). WGCNA identified three modules of genes
DURABLE HIV-1 ANTIBODY PRODUCTION IN HUMANS AFTER AAV8-MEDIATED GENE TRANSFER

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Background: Gene transfer protocols offer an alternative to repeated injections of HIV broadly neutralizing antibodies (bNAb) as a means of maintaining effective immunoprophylaxis. VRC07 is a bNAb targeting the CD4 binding site of the HIV-1 envelope glycoprotein.

Methods: Eight HIV-infected volunteers on effective ART therapy, age 30-60 yr, were enrolled in a phase I, open-label dose escalation trial of an AAV8 vector encoding the HIV bNAb VRC07 at doses of 5x10¹⁰ (N=3), 5x10¹¹ (N=2), and 2.5x10¹² (N=3) viral genomes per kilogram (vg/kg) by IM injection. All volunteers in the 5x10¹⁰ and 5x10¹¹ vg/kg doses were followed for 2 yr. Three volunteers in the 2.5x10¹² dose group have been followed for 4 yr.

Results: Product administration was well tolerated. No serious adverse events were attributed to product. Peak VRC07 concentrations were 0.17-0.43 μg/ml in the 5x10¹⁰ dose group, 0.25-0.74 μg/ml in the 5x10¹¹ dose group and 1.1-1.2 μg/ml in the 2.5x10¹² dose group. The data from 5 of the 8 volunteers suggest a pattern of antibody production defined by an early peak in VRC07 concentration followed by a secondary increase in concentration after 16 wks. In 4 of these 5 volunteers VRC07 concentration either increased or remained stable for >1 yr. In the 3 volunteers who did not show a secondary increase in VRC07 production, anti-VRC07 antibodies (ADA) were detected. In each case anti-VRC07 antibodies bound both VRC07 and the VRC07 Fab fragment. No correlation between the subject heavy chain IgG1 allotype and presence of ADA was found. After protein A Igg purification, in vitro IgG containing VRC07 was characterized. Measured VRC07 closely correlated with neutralization activity. In the 7 individuals where IgG containing VRC07 was characterized, pseudovirus neutralization IC 80s for 5 tier 2 pseudovirus were similar to reported IC80s for ex vivo produced VRC07. Neutralization of pseudovirus infections by purified IgG containing in vivo produced VRC07 was inhibited by the VRC07 paratope binding antibody SDR.

Conclusion: AAV vectors can safely be used to stably produce biologically active HIV-1 specific bNAbs in humans for over 1-year. AAV8 mediated gene transfer offers a means of generating vectored immunoprophylaxis in humans but the reasons for the induced ADA responses need to be understood to optimize future gene transfer protocols.

A PLACEBO-CONTROLLED ATI TRIAL OF HTI VACCINES IN EARLY TREATED HIV INFECTION

Lucia Bailon1, Anuska Llano2, Samandhy Cedeño2, Miriam B. Lopez1, Yovaninna Alarcon1, Pep Coló1, Angel Rivero1, Anne R. Lesebam1, Ian McGowan2, Devi SenGupta2, BonaventuraCotet1, Christian Brander1, Jose Molto2, Beatriz Mothe2, for the AELIX-002 Trial Group

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Background: HTI is a novel HIV vaccine immunogen designed at redirecting cellular immune responses to HIV targets associated with viral control.

Methods: The AELIX-002 trial (NCT03204617) was a randomized, single-center, placebo-controlled trial to evaluate the safety, immunogenicity and antiviral effect of DNA-HTI (D), MVA-HTI (H) and ChAdOx1-HTI (C) vaccines after discontinuation of ART in early-treated people living with HIV (PLWH). 45 participants were randomized (2:1) to receive heterologous prime-boost vaccination regimens consisting of DDDMM followed by CCM, or matched placebo (P). During a 24-week analytical treatment interruption (ATI), plasma viral load (pVL) was monitored weekly and ART was resumed if pVL >100,000 copies/ml, or >10,000 copies/ml over 8 weeks, and/or CD4<350.

Results: A total of 45 participants received DDDMM (n=30) or PPPP (n=15). Of the 45 participants, 41 further completed the CCM (n=26) or PPP (n=15) regimen and entered the ATI. Immunizations were well tolerated, with no SAFs, and were immunogenic in 97% of vaccine recipients (defined by a >2-fold increase in HTI-specific T cell responses compared to baseline). Median (range)
increase in total frequencies of HTI-specific T cells from baseline was 1,499 (120 to 3,150) SFC/million PBMC. At time of ART start, 71% (0 to 100) of the total anti-HIV-1 T-cell response was HTI-specific. For participants without any potentially beneficial HLA class I alleles (32 of the 41), 8 (40%) of the vaccines and 1 (8%) of the placebo recipients were able to remain off ART for 22 weeks (8.32%, 80.00% (7.6, 55.7)); with pVL <2,000 copies/mL being observed in 5 and 1 vaccine and placebo recipients, respectively. Magnitude of HTI-specific responses at the time of ART start positively correlated with time off ART in vaccinees (Rho 0.65, p < 0.01). Decay in total or intact HIV proviral DNA from baseline to ATI was similar between vaccine and placebo arms.

Conclusion: HTI vaccines were safe and highly immunogenic in early-treated PLWH with a prolonged time off ART seen in vaccinees with non-beneficial HLA class I alleles. Time off ART positively correlated with vaccine-induced HTI-specific T cell responses at ATI start. Multivariable analysis for other correlates of response is ongoing. These encouraging data strongly support the use of HTI-based vaccines as the backbone of combination cure regimens such as with the TLR7 agonist vesatolimum, which is currently being evaluated in the AELIX-003 study (NCT04364035).

162 NEURON DAMAGE AND RESERVOIR ARE SECONDARY TO HIV TRANSCRIPTS DESPITE SUPPRESSIVE ART

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Background: HAND persists despite suppressive ART for reasons that are unclear. We have previously shown that transcription without whole virus may explain HAND and contribute to the CSF HIV reservoir. Here we confirm and extend these findings with double the number of patients using our highly sensitive Double-R assay of HIV RNA/DNA and flow cytometry for cellular origin.

Methods: DNA and RNA were extracted from cells in 20 paired samples of CSF and blood taken from HIV-1 subjects (4 with current HAND and 2 with past HAND) on ART with both plasma and CSF HIV RNA (Roche) <50 copies/mL. HIV-1 transcripts and DNA levels were determined by the previously described Double-R assay on the extremely sensitive nCode MicroDics platform. Immunological profiles of CSF cells and PBMC were compared by 18-colour flow cytometry. In vivo brain injury was assessed with MR spectroscopy in the frontal white matter (FWM), posterior cingulate cortex (PCC), and caudate area.

Results: 18/20 patients’ CSF CD4+ T cells had significantly higher levels of cell-associated HIV-1 RNA transcriptional activity vs PBMCs (median 8,331 copies/106 CD4+ T cells, vs 680; p < 0.0001), with significant correlation of transcriptional activity within CD4+ T cells between CSF and PBMC (r = 0.46; p < 0.029). 16/20 patients also had significantly higher HIV-1 DNA levels in CSF CD4+ T cells (median 3,940 copies/106 T cells vs 885; p < 0.0001). CSF transcriptional activity was inversely correlated with the neuronal integrity biomarker N-acetyl aspartate (NAA) in FWM (p = 0.04) and PCC (p = 0.055). Transcriptional activity in PBMCs showed similar results: NAA in FWM (p = 0.051) and in PCC (p = 0.047). CSF cells were 91% memory T cells, with roughly equal memory CD4 (median 3,605 cells) and CD8 T cells (3,507 cells). Other CSF cells were 3.1% CD14+ CD16+ monocytes, 2.0% NK cells and 0.4% B cells. CXCR3+CD49d+ integrinβ7- cells were 76% of CSF CD4+ T cells compared with 17% of CD4 in PBMC; 51% were CCR5+ (vs 16% in PBMC); and 18% expressed CD38 and/or HLA-DR activation markers (vs 11% in PBMC).

Conclusion: CSF is an HIV reservoir with high transcription activity despite ART. It is biologically significant because of compromised neuron integrity likely mediated by transcription products (tat). The cellular source of HIV RNA is most likely the predominant CXCR3+ CD49d+ integrinβ7- CCR5+ memory CD4+ T cells; monocytes may be less important. Therapies targeting transcription should be developed.

163 3T BRAIN MRS REVEALS DISTINCT METABOLITE PATTERNS OF ART INITIATION DURING ACUTE HIV

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Background: Our previous studies have documented cellular neuroinflammation in acute HIV infection (AHI) and normalization after six month of early initiation of ART measured by 1.5T MRI single voxel proton magnetic resonance spectroscopy (1H MRS), specifically in the gray matter (basal ganglia, occipital gray, and frontal gray). We now explore the impact of early ART on white matter brain metabolism in AHI after two years of treatment using a 3T MRI, discriminating by variable timing of treatment initiation.

Methods: Thai acute HIV (AHI) participants enrolled in Bangkok, Thailand as part of the SEARCH 010/RV254 study completed 8-cc single voxel 1H MRS in the left frontal white matter (FWM) on a 3T MR scanner prior to ART onset (M0) and again after two years of ART (M24) initiated in Fiebig I–IV AHI. HIV negative (HC) participants were included for comparison. Brain metabolite levels were corrected for T1, T2, and CSF contribution within the MRS voxel. GraphPad Prism was used for statistical analysis. Two-tailed paired parametric T-tests were used to compare MRS outputs at baseline and two years after ART with Bonferroni correction.

Results: Thirty-seven male participants were categorized as Fiebig I–IV. There were no significant differences between brain metabolites at M0 compared to HC and AHI after ART (Table 1). At M0, participants with Fiebig I/II exhibited significantly lower tNAA, a marker of neuronal health, compared to HC, and showed a trend to normalization at M24. Among participants with Fiebig III/IV, tNAA was reduced at M24 compared to M0. In Fiebig I/II choline, the inflammation marker, was elevated at M24 compared to M0. Similar results were observed in Fiebig III/IV, where choline and myo-inositol were reduced at M24 compared to M0.

Conclusion: Participants with AHI that are in Fiebig stage III/IV show further brain metabolites declined compared to HC in white matter particularly cell generation markers, inflammation and glial dysfunction (choline, NAA, and myo-inositol) concurrent with the early initiation of ART in AHI after 2 years of treatment. Using the 4th generation immunomassay (Fiebig stages) to classify acute HIV infection may be beneficial measuring changes in frontal white matter brain metabolites levels after two years of ART.

### Table 1: Metabolite Levels in Fiebig and MRS Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (IQR)</th>
<th>p value</th>
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<tr>
<td>tNAA</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
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<td>Choline</td>
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<tr>
<td>Myo-inositol</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>NAA</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>PCr</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
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<tr>
<td><strong>PIBM</strong></td>
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<tr>
<td>tNAA</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>Choline</td>
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<tr>
<td>Myo-inositol</td>
<td>0.00 (0.00)</td>
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</tr>
<tr>
<td>NAA</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>PCr</td>
<td>0.00 (0.00)</td>
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### Table 2: Summary of Metabolite Levels

<table>
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<tr>
<th>Metabolite</th>
<th>Median (IQR)</th>
<th>P value</th>
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<tbody>
<tr>
<td>tNAA</td>
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<td>&lt;0.0007</td>
</tr>
<tr>
<td>Choline</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>0.00 (0.00)</td>
<td>&lt;0.0007</td>
</tr>
</tbody>
</table>
164 EFFECTS OF HIV AND AGING ON FUNCTIONAL CONNECTIVITY AND ANATOMY

Patrick H. Luckett1, Kayla Hannon1, John J. Lee1, Robert Paul2, Joshua S. Shimony1, Karin L. Meeker1, Sarah Cooley1, Beau M. Ances1
1Washington University in St Louis, St Louis, MO, USA, 2University of Missouri St Louis, St Louis, MO, USA

Background: The effects of HIV infection and aging on brain structural and functional connectivity and neuropsychological performance (NP) remains poorly understood. Understanding the effects of the virus using a lifespan perspective is vital for providing appropriate care to people living with HIV (PLWH).

Methods: 297 virologically well-controlled (<200 copies/mL) PLWH (mean age 48.5y, 67% male) on CART and 1509 HIV uninfected (HIV-) controls (mean age 45.4y, 62% male) matched for age, sex, and education were evaluated. All participants completed structural and functional neuroimaging. PLWH were classified as cognitively normal (HIVCN) or impaired (HIVCI) based on NP battery consisting of five domains. Relief feature selection identified the strongest predictive functional resting state networks (RSNs) with regards to HIV serostatus and degree of cognitive impairment within specific age bins (<35, 35-55, and >55 years old). Deep learning models identified where the largest structural differences occurred in relation to the identified RSNs.

Results: The Relief algorithm identified the strongest predictive RSNs of HIV status between HIV- controls and HIVCN as the salience (SAL) and parietal memory network (PMN). The strongest predictive RSNs of HIV status between HIV- controls and HIVCI were the SAL, PMN, and frontal parietal (FPN). The strongest predictive RSNs of HIV cognitive impairment status between HIVCN and HIVCI were the SAL, FPN, and ventral attention (VAN). When evaluating different age bins, FPN became a stronger predictor with age, SAL and VAN lost predictive strength, and PMN remained consistent. With structural neuroimaging, the primary differences in RSN topology occurred in cortical and subcortical regions, including the dorsal and rostral lateral prefrontal cortex (Figure 1), anterior cingulate, and caudate.

Conclusion: We have identified RSNs that discriminated HIV-, HIVCN, and HIVCI using machine learning. Our results suggest PMN and SAL are highly impacted by HIV, and additional involvement of FPN leads to increased cognitive impairment. Deep learning models identified where the largest differences in RSN topology occurred. When evaluating different age bins, variability in RSNs predictive strength was observed, even under viral suppression. These results suggest a complex set of RSN and structural changes that are unique to HIV status, aging, cognitive impairment status, and anatomy.

165 DIVERGENT AND SELF-REACTIVE IMMUNE RESPONSES IN THE CNS OF COVID-19 PATIENTS

Eric Song1, Christopher Bartley2, Ryan Chow1, Thomas Ngo2, Ruoyi Jiang1, Colin Zamecnik3, Ravi Dandekar2, Lindsay McAlpine1, Serena S. Spudich1, Joseph DeRisi2, Akiko Iwasaki1, Samuel Pleasure2, Michael Wilson2, Shelli F. Farhadian1, for the Yale IMPACT Team
1Yale University, New Haven, CT, USA, 2University of California San Francisco, San Francisco, CA, USA

Background: One third of COVID-19 patients develop significant neurological symptoms, yet SARS-CoV-2 is rarely detected in central nervous system (CNS) tissue, suggesting a potential role for parainfectious processes, including neuroimmune responses.

Methods: We examined immune parameters in CSF and blood samples from a cohort of hospitalized patients with COVID-19 and significant neurological complications (n=6), compared to SARS-CoV-2 uninfected controls (Fig1A). Intrathecal antibodies were assessed for anti-viral and auto-reactivity by ELISA, mouse brain immunostaining, phage display, and IP-MS.

Results: We found divergent intrathecal humoral response to SARS-CoV-2. Indeed, all COVID-19 cases examined had anti-SARS-
CoV-2 IgG antibodies in the CSF whose target epitopes diverged from serum antibodies. Next, we directly examined whether CSF resident antibodies targeted self-antigens and found a significant burden of CNS autoimmunity, with the CSF from most patients recognizing neural self-antigens. COVID-19 CSF produced immunoreactive staining of specific anatomic regions of the brain including cortical neurons, olfactory bulb, thalamus, and cerebral vasculature. Finally, we produced a panel of monoclonal antibodies from patients’ CSF and peripheral blood, and show that these target both anti-viral and anti-neural antigens—indicating one CSF-derived mAb specific for the spike protein that also recognizes neural tissue (Fig ID).

Conclusion: This immune survey reveals evidence of a compartmentalized and self-reactive immune response in the CNS in COVID-19 patients with neurologic symptoms. We identified both innate and adaptive anti-viral immune responses, as well as humoral autoimmunity that appears to be unique to the CNS during SARS-CoV-2 infection. These data suggest a potential role for autoimmunity in contributing to neurological symptoms, and merit further investigation to the potential role of autoantibodies in post-acute COVID-19 neurological symptoms.

Figure: A. Study design. B. Hierarchical clustering of cytokines increased (yellow) in the CSF (top) and plasma (bottom) of COVID-19 patients (purple) compared to controls (green). C. CSF of COVID-19 patients contains an increased frequency of B cells when compared to CSF from controls. D. Mouse brain immunostaining of monoclonal antibodies generated from COVID-19 patient CSF demonstrating reactivity to brain tissue, mAb2 is also reactive against the SARS-CoV-2 spike protein.

166 CHROMOSOMAL COPY NUMBER ALTERATIONS IN ANAL PRECANCERS FROM PEOPLE WITH HIV

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1Icahn School of Medicine at Mount Sinai, New York City, NY, USA
2University of Zagreb, Zagreb, Croatia, 3Icahn School of Medicine at Mount Sinai, New York City, NY, USA

Background: People living with HIV (PWH) are susceptible to high-risk human papillomavirus (HPV) infection of the anal canal owing to their immunocompromised status. The virus can transform anal squamous epithelium to low-grade squamous intraepithelial lesions (LSILs) and further to high-grade squamous intraepithelial lesions (HSILs). HSILs are well-defined cancer precursors that can progress to invasive cancer if left untreated. HPV-associated cancers commonly carry genomic abnormalities, specifically, a gain of chromosome 3q26 (PKI3CA), 20q13, 5p15 (TERT) and 7 centromere (cen7).

Methods: Anal lesions from 63 unique patients (36 HSIL, 27 LSIL) were obtained via high-resolution anoscopy (HRA)-directed biopsy. Anal swabs were aimed at the time of HRA to collect samples for cytological diagnosis and HPV DNA testing of HPV16, 18, and other (12) high-risk types. FISH-based HPV-associated Cancer Test (FHACT) was performed on the biopsy samples using four-color probes to detect any gain of chromosome 3q, 20q, 5p, and 7.

Results: Four-color probes detected any gain of chromosome 3q26, 20q13, 5p15, and 7 in 33% (30/91), 9% (8/91), 7% (6/91), and 0% (0/91), respectively. Genomic abnormalities were significantly more frequent in lesions associated with HPV16/18 infection, compared with those associated with non-16/18 types and negative HPV (42% vs. 24% vs. 9%; p=0.002). HSILs had a gain of 3q26, 20q13, 5p15, and cen7 in 42%, 31%, 31%, and 19% of HSILs and 7%, 4%, 0%, and 0% of LSILs, respectively. Genomic abnormalities were more frequent in lesions associated with HPV16/18 infection, compared with those associated with non-16/18 types and negative HPV (42% vs. 24% vs. 9%; p=0.002). The most common abnormality was the amplification of chromosome 3q, the location of PKI3CA gene. Our results suggest that PI 3-kinase/AKT signaling pathway may play an important role in anal cancer development in PWH.

<table>
<thead>
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<th>Table: Characteristics by Lesion Grade</th>
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<tr>
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<tr>
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<td>LSIL n=27</td>
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<tr>
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<tr>
<td><strong>AN3</strong></td>
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<td>Other HR HPV</td>
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1AIN: Anal intraepithelial neoplasia; HR HPV: High-risk human papilloma virus; HSIL: High-grade intraepithelial lesion; LSIL: Low-grade intraepithelial lesion.

167 POMALIDOMIDE AND LIPOSOMAL DOXORUBICIN FOR KAPOSI SARCOMA +/− OTHER KSHV DISEASES

Ramya Ramaswami1, Kathrym Lurain1, Anaïda Widell2, Priscilla Gonzalves1, Irene Ekwede1, William D. Figg1, Cody Peer1, Ralph Mangusian3, Jomy George1, Seth Steinberg1, Denise Whitby4, Thomas S. Udrick3, Robert Yarchoan5

1National Cancer Institute, Bethesda, MD, USA, 2Leidos Biomedical Research, Inc, Frederick, MD, USA

Background: Kaposi sarcoma herpesvirus (KSHV, also known as human herpesvirus 8 [HHV-8]), is the causative agent of Kaposi sarcoma (KS), a multicentric angio proliferative tumor, and other diseases including a form of multicentric Castleman disease (KSHV-MCD) and KSHV inflammatory cytokine syndrome (KICS). KS can be difficult to treat when it occurs with KSHV-MCD or KICS; resulting in high mortality rates. Liposomal doxorubicin (dox), a chemotherapy, and pomalidomide (pom), an immunomodulatory drug, are FDA-approved therapies for KS. The safety and activity of the combination (pom/dox) in KS alone or with KSHV-associated diseases are unknown.

Methods: The primary objective of this Phase I/II study was to evaluate safety and tolerability of pom/dox in 2 groups of participants with KS requiring systemic therapy: Group I (G1) - KS alone; Group II (G2) - KS with concurrent KSHV-MCD or KICS. Participants received dox at 20 mg/intravenously on day 1 of a 28-day cycle combined with pom once daily on days 1 to 21 at escalating dose levels (DL) (1 - 2mg, II - 3mg, or III- 4mg) in a 3+3 design until plateau of response or other pre-specified criteria. Participants received 81mg of aspirin daily as thromboprophylaxis. KS responses were evaluated using the modified AIDS Clinical Trial Group criteria.

Results: Thirty-four cisgender men, all with severe (T1) KS (21 patients (62%) in G1 and 13 patients (38%) in G2) were treated; 32 (94%) were HIV-infected and 22 (65%) had prior chemotherapy for KS (15/21 G1 and 7/13 G2). All participants
168 VAGINAL PH PREDICTS CERVICAL INTRAEPITHELIAL NEOPLASIA-2 REGRESSION IN HIV + WOMEN


Background: We previously reported that persistence/progression of cervical intraepithelial neoplasia-2 (CIN2) was uncommon in women living with HIV (WLH) from the Women’s Interagency HIV Study (WIHS, now MWCCS). Here we examined additional factors that may influence CIN2 natural history.

Methods: A total of 337 samples from 94 WLH with a confirmed CIN2 diagnosis were obtained from the MWCCS. 42 cervicovaginal HPV types and 34 cervicovaginal cytokines/chemokines were measured at CIN2 diagnosis (94 samples) and 6-12 months prior to CIN2 diagnosis (79 samples). Covariates, including CD4 count and vaginal pH, were abstracted from core MWCCS visits. Logistic regression models were used to explore CIN2 regression (CIN1, normal) vs. persistence/progression (CIN2, CIN3). Log rank tests, Kaplan Meier method, and Cox regression modeling were used to determine CIN2 regression rates.

Results: Participants were predominantly African American (53.2%) and using antiretroviral therapy (66.0%). The most prevalent HPV types across all visits were HPVs54 (21.6%) and 53 (21.3%); most prevalent high-risk (hr)-HPV types were 58 (18.4%) and 16 (17.5%). 33 women (35.1%) with incident CIN2 had a subsequent CIN2/CIN3 diagnosis during the study period (median 12.5 years of follow-up). Women who regressed had a higher CD4 T cell count at CIN2 diagnosis (Mann Whitney p=0.02). Each additional hr-HPV type detected at the pre-CIN2 visit was associated with increased odds of CIN2 persistence/progression (OR 2.27, 95% CI 1.15, 4.50). Higher vaginal pH (aOR 2.27, 95% CI 1.15, 4.50) and bacterial vaginosis (aOR 5.08, 95% CI 1.30, 19.94) at the CIN2 diagnosis visit were associated with higher odds of CIN2 persistence/progression (OR 2.46, 95% CI 1.19, 5.13). Adjusting for CVL storage time, self-reported STI status, or CVL contaminants did not affect results.

Conclusion: We found relatively low prevalence of HPV16/18 in this cohort. Elevated vaginal pH at the time of CIN2 diagnosis may be a useful indicator of CIN2 persistence/progression and the rate of persistence/progression.

169 WILMS’ TUMOR 1 IS OVEREXPRESSED IN KAPOSI SARCOMA AND IS REGULATED BY VFLP/K13

Ayana Morales, Cailyn Genoves, Matthew Bott, Julio Alvarez, Sung Soo Mun, Jennifer Tontony, Jesus Delgado, Stephanie Chang, Maite Ibáñez de Gaya, David Scheinberg, Paul Rubinstein, Thomas Campbell, Margaret Borok, Susan Krown, Ethel Gesaran

Background: Kaposi Sarcoma (KS), a vascular neoplasm caused by Kaposi sarcoma herpesvirus (KSHV, also called HHV-8), is the most common HIV associated malignancy globally. A National Cancer Institution (NCI) Prioritization Summit ranked Wilms’ Tumor 1 (WT1) as the #1 tumor associated antigen to direct immunotherapy towards. Various forms of WT1 immunotherapies are in development. Following on our early data showing WT1 upregulation in KS in 46 cases (CROI 2019), we assessed WT1 expression in KS in an expanded cohort, and determined if KSHV infection accounts for this upregulation. Among the KS+ genes, we focused on vFLIP because of its ability to induce NF-κB, a transcription factor known to affect WT1 levels.

Methods: We used immunohistochemistry analysis to evaluate 303 biogies of advanced HIV associated KS from clinical trial AMC066/AS263 for expression of WT1, LANSA, the presence of CD4+ and CD6+ T cells. We examined effects of KSHV on WT1 expression in vitro using endothelial cell infection models, and determined whether a single latent viral gene, vFLIP, influences WT1 expression. We knocked down WT1 using WT1 lentiviral shRNA. We then tested a T cell receptor mimic antibody, ES:K-1, specific for WT1 peptide/HLA-A02 expression, for its ability to bind KSHV-infected or vFLP-expressing endothelial cells

Results: Moderate to strong WT1 expression (>20% WT1 positive cells) was found in 64.9% of the 303 biopies, and in 92.3% of nodular lesions. WT1 expression correlated with increased histopathologic stage, expression of the viral latent oncoprotein (LANA); r=0.687, p=0.0001), and was inversely correlated with the quantity of CD8+ T cells (r=−0.2536 p=0.0001). In vitro, KS+ endothelial cells expressed WT1, and KSHV infection of endothelial cells resulted in upregulation of WT1. We found that vFLIP expression alone upregulates WT1 and appears dependent on NF-κB. Flow cytometry using ES:K-1 antibodies, showed increased binding to endothelial cells with KSHV infection or vFLIP expression compared to mock infected or uninduced endothelial cells. WT1 loss in KSHV-infected endothelial cells in vitro appears to associate decreased vFLIP and LANSA expression.

Conclusion: WT1 is overexpressed in Kaposi sarcoma and is upregulated by vFLIP/K13. Our data demonstrate increased binding of WT1 overexpressing endothelial cells by ES:K-1, a human monoclonal antibody in preclinical development as adjuvant immunotherapy directed towards WT1 overexpressing malignancies. Immunotherapy directed towards WT1 may prove as a new treatment strategy in KS.

170 INFILTRATING TUMOR-ASSOCIATED MACROPHAGES IN KAPOSI SARCOMA-ASSOCIATED DISORDERS

Ramy Ramaswami, Silvia Lage, Kathryn Curran, Joseph Rocco, Maura Manion, Robert Varchan, Irini Seeti

Background: Kaposi sarcoma herpesvirus (KSHV) causes several disorders: Kaposi sarcoma (KS), primary effusion lymphoma (PEL), a plasmablastic form of multicentric Castleman disease (MCD) and most recently inflammatory cytokine syndrome (KCS), which may occur alone or concurrently in the same patient. These conditions occur among people living with HIV. KSHV-associated disorders (KAD) are characterized by elevated plasma levels of inflammatory mediators, resulting in systemic symptoms, which if not identified and treated can result in high mortality. Activation of the inflammasome, which leads to...
pro-inflammatory cytokines such as IL-1β and IL-18 via active caspase-1, has not been assessed in patients with KAD.

**Methods:** Peripheral blood mononuclear cells (PBMCs) from 9 healthy controls (HCs) and 33 participants with KAD (PEL (5), MCD (9), KS (6), KICS (6) or overlap of these conditions (7)) were incubated with a probe to assess active caspase-1 (FLICA) followed by staining for ASC (apoptosis associated speck like protein containing CARD) specks in peripheral monocytes to detect inflammasomes with active caspase-1. T-test and Wilcoxon rank sum tests were used to assess differences in speck formation and plasma cytokine levels between HC and KAD participants.

**Results:** Samples were obtained from 33 cisgender HIV-infected men with KAD (median [med] age 44 years; med CD4 count: 250 cells/µL; med HIV VL <50 copies/mL) and 9 HCs. KAD participants had higher IL-18 plasma levels than HCs (median: 2595 vs. 794 pg/mL; p<0.0009), indicating that KAD are accompanied by systemic inflammasome activation. We also found higher levels of monocytes demonstrating spontaneous FLICA+ ASC-speck formation in those with KAD compared to HCs (KAD: 89,546 cells/mL, 4.60% vs HCs: 30,965 cells/mL, 1.39%, p=0.0002 and p<0.001, Figure 1). While there were higher levels of classical CD14highCD16- monocyte subsets in the majority of KAD participants, those with MCD alone and PEL alone also had evidence of FLICA+ ASC-specks inside the intermediate (CD14highCD16-) and patrolling (CD14lowCD16+) monocyte subsets, respectively. Overall, as compared to participants with one KAD, those with more than one KAD did not have significantly different monocytes with FLICA+ ASC-specks/mL (p=0.09).

**Conclusion:** Our data suggest that activation of the inflammasome in circulating blood monocytes contributes to the pathogenesis of KSHV-associated disorders and could potentially represent a target for host-directed therapy against these diseases.

**171 COVID-19 INFECTION IN HIGH-RISK SOUTH AFRICAN PREGNANCIES WITH AND WITHOUT HIV**

Liesl De Waard1, Eduard J. Langenegger1, Kobie Erasmus1, Tian Van der Merwe1, Nicole Du Toit1, Chane Paulsen1, Nontando S. Nkangana1, Susanna E. Olivier1, Sonja Schell1, Magriet Van Niekerk1, Jantjie J. Taljaard1, Angela Dramowski1, Cathy A. Cluver1, Adrie Bekker1

1Stellenbosch University, Tygerberg, South Africa

**Background:** Data from Africa reporting the outcomes of COVID-19 infection in pregnancy are limited, particularly for women with high-risk pregnancies (hypertension, diabetes and obesity) and pregnant women living with HIV (PLHIV). We describe the clinical features, maternal and birth outcomes of COVID-19 high-risk pregnancies at a South African tertiary care referral hospital with a 24% antenatal HIV prevalence.

**Methods:** We prospectively collected data from COVID-19 infected pregnant women attending the high-risk obstetric service at Tygerberg Hospital, Cape Town, between 1 May 2020 and 31 July 2020, and documented pregnancy and birth outcomes until 30 October 2020. Laboratory testing for SARS-CoV-2 infection was performed only in symptomatic pregnant women. Descriptive analysis was performed for all COVID-19 infected women with high-risk pregnancies; demographic and outcome variables were compared for PLHIV versus pregnant women without HIV.

**Results:** One hundred pregnant women (72 without HIV and 28 PLHIV) had laboratory-confirmed COVID-19 infection (Table 1). Obesity, hypertensive disorders and gestational diabetes were frequent comorbidities. Among 28 PLHIV, the majority received antiretroviral treatment 27 (96%); median CD4 count was 441 (14-838) cell/mm³ for 21 (75%) and 19 (73%) were virologically suppressed. COVID-19 infection was diagnosed predominantly in the 3rd trimester (81%); 50% of women delivered within 2 weeks of infection onset. Forty women developed pneumonia; 13 developed adult respiratory distress syndrome (ARDS) and 6 required invasive ventilation. Eight women died, 7 from ARDS and 1 from advanced HIV disease with bacteremia. Pregnancy outcomes included 91 live births (including 5 sets of twins), 5 stillbirths, 4 miscarriages, 2 mothers who died with the fetus in situ and 1 medical termination of pregnancy. Birth outcomes for 2 women were unknown. Outcome for the 91 liveborn neonates were good except for one who died from complications related to perinatal asphyxia. No significant differences for COVID-19 infection impact and outcome were noted for PLHIV versus those without HIV.

**Conclusion:** In this cohort of high-risk pregnant women with COVID-19 infection, no clinical differences in outcome attributable to HIV-infection were noted, however the majority of PLHIV were virally suppressed. The impact of COVID-19 infection in pregnancy was severe (40% complicated by pneumonia; 8% crude mortality rate); neonatal outcomes were favourable.

| Table 1. Characteristics and outcomes of high-risk COVID-19 pregnancies at Tygerberg Hospital by HIV status (n=100) |
|----------------------------------|------------------|------------------|------------------|------------------|
| Age in years, median (IQR)       | 31 (27–35)       | 31 (27–37)       | 34 (31–39)       | 0.08             |
| Pre-existing medical conditions  |                  |                  |                  |                  |
| Chronic hypertension (%)         | 22 (22)          | 19 (26)          | 3 (11)           | 0.07             |
| Severe Obesity                   | 36 (34)          | 28 (39)          | 0.22 (36)        | 0.07             |
| Obstetric complications          |                  |                  |                  |                  |
| Gestational hypertension         | 18 (19)          | 13 (23)          | 5 (18)           | 0.98             |
| Pre-eclampsis                    | 24 (24)          | 18 (22)          | 6 (21)           | 0.70             |
| Gestational diabetes             | 14 (14)          | 12 (17)          | 2 (7)            | 0.18             |
| COVID-19-related complications   |                  |                  |                  |                  |
| Pneumonia (%)                    | 40 (40)          | 27 (38)          | 13 (47)          | 0.41             |
| ARDS (%)                         | 13 (13)          | 8 (11)           | 5 (18)           | 0.28             |
| ICU admission (%)                | 17 (17)          | 9 (13)           | 6 (21)           | 0.35             |
| Outcomes                         |                  |                  |                  |                  |
| Live births (%)                  | 91 (91)          | 66 (92)          | 25 (89)          | 0.58             |
| Maternal mortality (%)           | 8 (8)            | 6 (8)            | 2 (7)            | 0.60             |

IQR, interquartile range; BMI, body mass index; ARDS, Adult Respiratory Distress Syndrome; ICU, intensive care unit

**172 CHARACTERISATION OF SARS-CoV-2-INFECTED CHILDREN DEVELOPING NEUTRALISING ANTIBODIES**

Alessandra Ruggiero1, Nicola Cotugno1, Bonfante Francesca2, Maria Raaffella Petrara1, Giuseppe R. Pascucci1, Veronica Santilli1, Emma Manno1, Carlo Concato1, Giulia Linardos1, Daniela Dona1, Carlo Giaguinto1, Petter Brodin1, Paolo Rossi1, Anita De Rossi1, Paolo Palma1

1Bambino Gesu Children’s Hospital, Rome, Italy, 2Istituto zooprofilattico delle Venezie, Legnaro, Italy, 3University of Padova, Padova, Italy, 4University of Padova, Padova, Italy, 5Karolinska Institute, Stockholm, Sweden

**Background:** Further knowledge on adaptive immunity to SARS-CoV-2 (CoV-2) in children is needed in order to define possible immunization strategies and reconsider pandemic control measures. We analyzed anti-CoV-2 antibodies (Ab) and their neutralizing activity (PRNT), alongside antigen (Ag) specific cellular response, in relation to virus load in nasopharyngeal swabs.

**Methods:** We analysed 42 CoV-2 patients at 7 days after symptoms onset. CoV-2 viral load (VL) was measured by RT-PCR and digital droplet PCR on longitudinal samples of nasopharyngeal swabs (NP). Virus infectivity (FFU) in children is needed in order to define possible immunization strategies and reconsider pandemic control measures. We analyzed anti-CoV-2 antibodies (Ab) and their neutralizing activity (PRNT), alongside antigen (Ag) specific cellular response, in relation to virus load in nasopharyngeal swabs.

**Results:** We analysed 42 CoV-2 patients at 7 days after symptoms onset. CoV-2 viral load (VL) was measured by RT-PCR and digital droplet PCR on longitudinal samples of nasopharyngeal swabs (NP). Virus infectivity (FFU) was tested by virus focus forming assay. CoV-2 antibodies were investigated by Disaron (CoV-2 Ab) and neutralization assay (PRNT). CoV-2-specific CD4-CD40L+ T-cells and Spike specific B-cells were analysed by flow cytometry. Plasma proteomic profiling was measured by 2 Olink panels. The area under the curve (AUC) of the viral load from NP collected every 48 hours up to undetectable VL. Mann-Whitney was used to compare means in individuals with clinical symptoms (PRNT+) or not (PRNT-); linear regression was used to evaluate the associations between virus load and infectivity over time. Principal component analysis (PCA) was used to analyse proteomic data.
response was associated with the presence of CoV-2 specific IgG-CD27+ B cells, with a higher frequency of CoV-2 specific B cells found in seropositive compared to seronegative (p=0.001). Besides, individuals developing neutralizing Ab had higher frequency CD4-CD40L+ T-cells compared to PRINT -p=0.03. The plasma proteome confirmed the association between cellular and humoral CoV-2 immunity, with PRINT showing higher viral signal transduction molecules (SLAMF1, CD244, CLEC4G).

Conclusion: This work provides a virological and immunological characterization of SARs-CoV-2 infected children presenting a differential Ab-mediated neutralizing activity. It demonstrates that children with neutralizing antibodies present reduced viral load, faster virus clearance and lower in vitro infectivity. These data provide information that can drive vaccination endpoints and quarantine measures policies.

173 PEDIATRIC AND ADOLESCENT HIV TESTING AND DIAGNOSIS IN THE CONTEXT OF COVID-19

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Background: In 2019, UNAIDS estimated there were 150,000 new HIV infections among children (<15 years old) and 170,000 among adolescents (10-19 years old), highlighting the ongoing need for HIV testing and diagnosis among these populations. We aim to describe the impact of COVID-19 on HIV testing and diagnosis in children and adolescents.

Methods: We analyzed U.S. President’s Emergency Plan for AIDS Relief (PEPFAR) Monitoring, Evaluation and Reporting (MER) data from 14 countries in sub-Saharan Africa to compare the number of children (1-14) and older adolescents (15-19) who received an HIV test and were diagnosed as HIV-positive before (January – March, 2020) and during (April – June, 2020) the COVID-19 pandemic across all HIV testing modalities and for index testing (i.e. exposure-based). We calculated the percent change for the two indicators in the two time periods.

Results: Overall, pediatric HIV testing and diagnoses declined by 40% and 29%, respectively, across the 14 countries. The testing decline ranged from -13% (DRC) to -81% (Zimbabwe) with the greatest volume of decline in South Africa (-150,469). Lesotho (-61%), Zimbabwe (-57%) and South Africa (-53%) had the largest declines in testing for this group with the greatest volume of decline in South Africa (-147,891). Seven countries had >25% declines in HIV diagnoses for older adolescents, with Lesotho (-50%) and Zimbabwe (-49%) having the largest declines. While index testing for older adolescents decreased in most countries (-31%), it increased in Cameroon (+25%), Nigeria (+20%) and Côte d’Ivoire (+15%).

Conclusion: Pediatric and adolescent HIV testing and diagnoses dramatically declined in many sub-Saharan African countries during the COVID-19 pandemic. Countries – like Cameroon, Côte d’Ivoire and Nigeria – that maintained or increased index testing during COVID-19 had the lowest declines in case finding. To mitigate the effects of COVID-19, programs may consider strategies to maximize index testing for children and adolescents (<19) of people living with HIV.

Table 1. Changes in HIV Testing and HIV-Positive Results for Children (1-14 years) and Older Adolescents (15-19 years) before (January – March, 2020) and after (April – June, 2020) COVID-19

174 DOLUTEGRAVIR-BASED ART IS SUPERIOR TO NONRTI/PPI-BASED ART IN CHILDREN AND ADOLESCENTS

Anna Turkova, for the ODYSSEY/PENTA-20 Trial Team

MRC Clinical Trial Unit at UCL, London, UK

Background: ODYSSEY is an international multi-centre randomised non-inferiority trial evaluating dolutegravir (DTG) + 2NRTIs versus standard-of-care (SOC) in children starting first- or second-line ART.

Methods: The primary outcome is a Kaplan-Meier estimated proportion of treatment failure defined as confirmed viral load (VL) >400c/mL after week 36, lack of virological response by 24 weeks with ART switch, death or new/ recurrent WHO4/severe WHO event by 96 weeks. Non-inferiority margin is 10% (12% for first-/second-line subgroups).

Results: 707 children ≥14kg were randomised (Uganda 47%, Zimbabwe 21%, South Africa 20%, Thailand 9%, Europe 4%); 350 to DTG; 357 to SOC. Median (range) age was 12.2 years (2.9-18); weight 31kg (14-85); 51% male. 311 children started first-line (92% efavirenz among SOC); 396 second-line (72% lopinavir/ritonavir, 25% atazanavir/ritonavir among SOC). Median follow-up was 142 weeks; 687 (97%) reached the primary endpoint or were seen on/after 96 weeks. 48 (14%) DTG vs 75 (22%) SOC had treatment failure by 96 weeks; difference (95% CI) –7% (-13.2, -2.3), p=0.006. 40 vs 67 were virological failures and 8 vs 8 were WHO3/4 events/death. Treatment effects were similar in first- and second-line, with no evidence of heterogeneity (p=0.20; fig). 13 (4%) children randomised to DTG changed regimen during follow-up vs 32 (9%) SOC (excluding NRTI changes and changes for growth, simplification, guideline change, switch-out) (p=0.004); 2 vs 21 changes were for treatment failure. At 48 and 96 weeks, proportion with cross-sectional VL<50c/mL and change in CD4 count from baseline were similar between arms. There were 65 SAEs (35 children) in DTG versus 46 (42) in SOC (p=0.45), including 2 vs 3 deaths; 119 (73 children) grade ≥3 adverse events in DTG vs 135 (88) in SOC (p=0.23). At week 96, mean change in total cholesterol from baseline was -5 mg/dL (95% CI -8,-2) in DTG versus 10 mg/dL (7,13) in SOC (difference (DTG-SOC) -15 (-19,-11); p<0.001). Weight, height and BMI increased marginally more in DTG than SOC (differences (SE) between arms 1kg (0.4), 0.7cm (0.3), 0.3kg/m² (0.1) respectively at 96 weeks).

Conclusion: DTG-based ART was superior to SOC based on treatment failure by 96 weeks in children/adolescents starting first- or second-line. There were no safety concerns on DTG. These results support WHO guidelines which recommend DTG-based regimens as preferred ART for children ≥14kg starting first- or second-line ART, allowing harmonisation with adult treatment programmes.
175 DolPHIN2 FINAL RESULTS DOLUTEGRAVIR VS EFAVIRENZ IN LATE PREGNANCY TO 72W POSTPARTUM

Thokozile R. Malaba, Irene Nakatudde, Kenneth Kintu, Tao Chen, Sabrina Bakeera-Kitaka, Lucy Read, Helen Reynolds, Angela Colbers, Kelly Byrne, Duolao Wang, Catriona Waitt, Catherine Orell, Mohammed Lamorde, Landon Myer, Saye Khoo

University of Cape Town, Cape Town, South Africa, Makerere University, Kampala, Uganda, Liverpool School of Tropical Medicine, Liverpool, UK, University of Liverpool, Liverpool, UK, Radboud University, Nijmegen, Netherlands

Background: Delayed ART initiation in pregnancy is associated with failure to achieve viral suppression and increased risk of MTCT. DolPHIN-2 (NCT03249181) randomized pregnant women initiating treatment in the third trimester to either dolutegravir (DTG) or efavirenz (EFV) based regimens in South Africa and Uganda. Preliminary analysis of the primary endpoint (viral load (VL) <50 copies at delivery) have been published.

Methods: Between Jan-Aug 2018, 268 mothers (safety cohort) were randomized to receive EFV (133) or DTG (135), of whom 250 (EFV-125, DTG-125, intention-to-treat cohort) were evaluable for efficacy. In addition to measurement in pregnancy, VL was also measured at 6, 12, 24, 48 and 72w postpartum (PP). The primary endpoints were VL<50 copies/ml for efficacy; and the occurrence of maternal and/or infant drug related serious adverse events (SAE) for safety. Here we present final data with follow-up of mothers and infants to 72w PP.

Results: As previously reported, DTG was associated with superior responses (VL<50) in the first 26w of therapy. At 72w, 116/125 mothers receiving DTG achieved VL<50 with a median time of 4.14 (IQR 4.00, 5.14) weeks. In contrast, among 114/125 mothers randomized to the EFV arm, suppression was achieved at a median time of 12.14 (IQR 10.71, 13.29) weeks (adjusted HR 1.93 (95% CI 1.47, 2.53) (figure). By 72w PP, 21.3% of mothers and 56.2% of infants experienced an SAE, however in mothers only 3% was related to study drug, with no infant drug related events. DTG was well tolerated with a lower frequency of maternal drug related AE (DTG 2.2% vs EFV 3.6%). Overall, the mean change in maternal weight from delivery to 72w PP was -1.2kg, with nonsignificant differences observed by arm in weight retention (DTG -0.7kg vs EFV -1.6kg). No differences in maternal glycosuria or infant hyperglycaemia were observed by arm. Overall 4 infant HIV infections were detected; 3 at delivery in DTG arm, with a new transmission detected at 72w PP in EFV arm despite optimal maternal suppression (VL<50) from delivery and serial negative tests in the child.

Conclusion: Maternal DTG-based ART was safe and well tolerated. Women randomized to DTG had more rapid viral suppression after initiation of ART and they maintained virologic suppression through the breastfeeding period. The infant HIV infection in the EFV arm highlights the potential for transmission during breastfeeding in mothers despite evidence of virologic suppression.

176 ANTEPARTUM WEIGHT GAIN AND ADVERSE PREGNANCY OUTCOMES IN IMPACT 2010

Risa M. Hoffman, Lauren Ziemba, Sean Brummel, Lameck Chinula, Teacler G. Nematadzira, Frances Nakayiwa, Jeff Stringer, Chelsea Krofte, Patrick Jean-Philippe, Anne Coletti, Rebecca Zash, Roger Shapiro, Paul Sax, Judith S. Currier, Shahn Lockman

University of California Los Angeles, Los Angeles, CA, USA, Harvard TH Chan School of Public Health, Boston, MA, USA, University of North Carolina Project–Malawi, Lilongwe, Malawi, University of Zimbabwe, Harare, Zimbabwe, Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, Frontier Science Ltd., Inverness-shire, UK, National Institute of Allergy and Infectious Diseases, Rockville, MD, USA, FHI 360, Durham, NC, USA, Beth Israel Deaconess Medical Center, Boston, MA, USA, Brigham and Women’s Hospital, Boston, MA, USA

Background: Insufficient and excess weight gain during pregnancy have been associated with adverse pregnancy outcomes. We evaluated the association between antepartum weight gain and adverse pregnancy outcomes in secondary analyses of IMPACT 2010 data.

Methods: Women with HIV-1 in 9 countries were randomized 1:1:1 at 14-28 weeks gestational age (GA) to start dolutegravin (DTG)+emtricitabine (FTC)/tenofovir alafenamide fumarate (TAF) vs. DTG+ FTC/tenofovir disoproxil fumarate (TDF) vs. efavirenz (EFV)/FTC/TDF. By-arm differences in average antepartum weekly weight gain were estimated using generalized estimating equations. Low weight gain was defined as <0.18 kg/week and high weight gain as >0.59 kg/week (Institute of Medicine). Time to event analyses were used to estimate risk of the composite adverse pregnancy outcome of stillbirth (≥20 wks GA), preterm delivery (<37 wks GA) and small for gestational age (SGA: <10th percentile), as well as each of these individual outcomes and neonatal death, using Cox-proportional hazards regression models with weight gain as a time-varying covariate.
Results: 643 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. The rate of adverse pregnancy outcome was lowest with DTG+FTC/TAF. Weekly average weight gain was highest with DTG+FTC/TAF (0.378 kg) compared to DTG+FTC/TDF (0.319 kg), p<0.011 and EFV/FTC/TDF (0.291 kg), p<0.001. Low weight gain was least common with DTG+FTC/TAF (15.0%) compared with DTG+FTC/TDF (23.6%) or EFV/FTC/TDF (30.0%), with the opposite pattern for high weight gain: DTG+FTC/TAF (2.7%) vs. DTG+FTC/TDF (9.9%) vs. EFV/FTC/TDF (6.3%). Overall, low weight gain was associated with higher risk of any adverse pregnancy outcome (HR 1.4, 95% CI 1.02, 1.96) and of SGA (HR 1.5, 95% CI 0.99, 2.22). For women in the DTG+FTC/TAF arm, low weight gain was also associated with higher risk of stillbirth (HR 6.2, 95% CI 1.16, 32.81) and preterm delivery (HR 3.7, 95% CI 1.14, 11.92) compared with normal weight gain. There were no associations between high weight gain and adverse pregnancy outcomes or low or high neonatal weight and neonatal death.

Conclusion: Low (but not high) weight gain was associated with adverse pregnancy outcomes. Women starting DTG+FTC/TAF in pregnancy gained more weight antepartum than women starting DTG+FTC/TDF or EFV/FTC/TDF, while women starting EFV/FTC/TDF had the lowest weight gain.

Results: We randomized 643 women: DTG+FTC/TAF (N=217), DTG+FTC/TDF (N=215), and EFV/FTC/TDF (N=211). Baseline medians: GA 21.9 weeks, HIV RNA 903 cp/mL, CD4 count 466 cells/μL. Six hundred and seven (94.4%) women and 566 (91.7%) of 617 liveborn infants completed the study; 479 (77.6%) infants breastfed (median 49.9 weeks). There were no apparent differences through week 50 PP between arms in the estimated probability of maternal grade≥3 AEs or infant grade≥3 AEs (Table 1). The average change in women’s weight from entry through PP was: -0.027 kg/week in DTG+FTC/TAF, -0.050 kg/week in DTG+FTC/TDF, and -0.080 kg/week in EFV/FTC/TDF arms. The estimated probability of infant death was higher in the EFV (6.9%) compared to DTG+FTC/TAF (1.0%, p<0.001) and DTG+FTC/TDF (2.0%, p=0.008) arms. Either stillbirth (previously reported) or infant death (combined) occurred as follows: 10 in DTG+FTC/TAF, 15 in DTG+FTC/TDF, and 18 in EFV/FTC/TDF arms. Four infants were diagnosed with HIV: 2 in DTG+FTC/TAF, 1 in DTG+FTC/TDF, and 1 in EFV arm. At 50 weeks PP, percent similarity of women with HIV RNA <200 cp/mL were similar in the combined DTG arms (96.3%) and EFV arm (96.4%). Regimen stops or switches were more frequent in the EFV arm due to virologic failure/drug resistance, and more frequent in the DTG arms due to PP fertility choices.

Conclusion: At week 50 PP, maternal and infant grade≥3 AEs from enrollment through week 50 PP were similar across arms; infant mortality was higher (though stillbirths somewhat less frequent) in those whose mothers were in the EFV/FTC/TDF arm. Maternal HIV RNA suppression was similarly high in the combined DTG vs the EFV arm, although more women stopped EFV due to virologic failure.

SAFETY/EFFICACY OF DTG VS EFV, TDF VS TAF IN PREGNANCY/POSTPARTUM: IMPAACT 2010 TRIAL
Lameck Chinula1, Sean Brumme1, Lauren Ziemba1, Katie McCarthy1, Benjamin Johnston2, Nahida Chakhtoura3, Patrick Jean-Philippe4, Lynda Stranix-Chibanda5, Violet Korutaro6, Haseena Cassim7, Shahin Lockman8, Judith S. Currier13, Lameck Chinula1, Haseena Cassim7, Shahin Lockman8, Judith S. Currier13, Lilangwe, Malawi, Harford TH Chan School of Public Health, Boston, MA, USA, FHI 360, Durham, NC, USA, 1Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA, 2National Institute of Child Health and Human Development, Bethesda, MD, USA, 3National Institute of Allergy and Infectious Diseases, Rockville, MD, USA, 4University of Zimbabwe, Harare, Zimbabwe, 5Baylar College of Medicine Children’s Foundation, Kampala, Uganda, 6Sweetove IMPAACT CRS, Johannesburg, South Africa, 7Wits Reproductive Health and HIV Institute, Johannesburg, South Africa, 8Botswana Harvard AIDS Institute Partnership, Gabarone, Botswana, 9Brigham and Women’s Hospital, Boston, MA, USA, 10University of California Los Angeles, Los Angeles, CA, USA

Background: We previously reported safety and virologic efficacy of dolutegravir (DTG)+emtricitabine (FTC)/tenofofam ide fumarate (TAF) vs DTG+ FTC/efavirenz (EFV) vs dolutegravir (EFV)/FTC/TDF delivery through week 50. Here we present results from enrollment through week 50 postpartum (PP).

Methods: Pregnant women with HIV-1 in 9 countries were randomized 1:1:1 to start open-label DTG+ FTC/TAF, DTG+ FTC/TDF, or EFV/FTC/TDF at 14-26 weeks gestational age (GA). Safety outcomes included pairwise regimen comparisons of grade≥2 maternal and infant adverse events (AEs, primary), infant mortality, and infant HIV infection. Safety probabilities were estimated with the Kaplan-Meier method. Efficacy analyses include comparison of maternal HIV RNA <200cp/mL at week 50 PP between the combined DTG arms and EFV arm.

Table 1: Impact of maternal/infant safety outcomes and maternal/neonatal efficacy outcomes through 50 weeks postpartum

Table 1

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<tr>
<th>Maternal outcomes through week 50 PP</th>
<th>Infant outcomes through week 50 PP</th>
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<tr>
<td>Maternal grade&gt;=3 adverse events (%)</td>
<td>Infant grade&gt;=3 adverse events (%)</td>
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<tr>
<td>Maternal death (%)</td>
<td>Infant death (%)</td>
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<td>Maternal stillbirth (%)</td>
<td>Infant stillbirth (%)</td>
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<td>Infant preterm birth (%)</td>
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<td>Maternal caesarean sections (%)</td>
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<th>Safety outcomes measured</th>
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<tr>
<td>Maternal grade≥3 AEs (%)</td>
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<td>Maternal preterm birth (%)</td>
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<td>Maternal caesarean sections (%)</td>
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<th>Efficacy outcomes measured</th>
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<tr>
<td>Maternal viral suppression (%)</td>
<td>Infant viral suppression (%)</td>
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<tr>
<td>Maternal HIV RNA &lt;200 cp/mL</td>
<td>Infant HIV RNA &lt;200 cp/mL</td>
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178 ADVERSE PREGNANCY OUTCOMES AMONG HIV-INFECTED WOMEN EXPOSED TO ISONIAZID IN BRIEF-TB
Amrita Gupta1, Michael Hughes2, Jorge T. Leon-Cruz2, Anchalee Avihingsanon7, Noluthando Mwelase8, Amita Gupta1, Laura Moran1, Constance A. Benson1, Richard E. Chaisson8, Susan Swindells9, for the ACTG 5279 BRIEF TB Trial
1The Johns Hopkins University, Baltimore, MD, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Thai Red Cross AIDS Research Center, Bangkok, Thailand, 4Wits Reproductive Health and HIV Institute, Johannesburg, South Africa, 5GSK SKOL, Port-au-Prince, Haiti, 6Botswana Harvard AIDS Institute Partnership, Gabarone, Botswana, 7Social & Scientific Systems, Silver Spring, MD, USA, 8University of California San Diego, San Diego, CA, USA, 9The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 10University of Nebraska Medical Center, Omaha, NE, USA

Background: Isoniazid (INH) preventive therapy (IPT), a key strategy for reducing tuberculosis (TB) and death among persons with HIV (PWH), was associated with increased composite adverse pregnancy outcomes in IMPAACT P1078 trial. The BRIEF-TB trial demonstrated non-inferiority of one-month versus nine-month isoniazid and rifapentine versus nine months of IPT. We assessed adverse pregnancy outcomes among women exposed to IPT in a secondary analysis.

Methods: BRIEF-TB enrolled 3,000 adults with HIV infection in 10 countries. Pregnancy was an exclusion criterion at entry. We analyzed adverse pregnancy outcomes (non-live births; a composite of still-births, spontaneous abortions, ectopic pregnancy; preterm; low birthweight) among women who became pregnant on IPT.
pregnant in the IPT arm, according to whether the first pregnancy during follow-up started during INH use (INH-exposed) or after IPT was finished (INH-unexposed). Logistic regression models were used to evaluate association of pregnancy outcome with INH exposure adjusting for age, CD4, antiretroviral therapy (ART) and latent TB infection (LTBI) status at study entry, and separately for these variables at or proximal to pregnancy outcome.

**Results:** Of 1,614 women participants, 812 were randomized to IPT, 136 of whom became pregnant; 128 of 136 had pregnancy outcome data available. At entry, median age was 29 years, median CD4 was 314 cells/mm$^3$, 45 (35%) were on ART (EFV or NVP-based regimen), and 26 (20%) were LTBI-positive. There were 93 live births and 35 non-live births (including 6 elective abortions). Sixteen (41%) of 39 INH-exposed pregnancies had non-live births (including 3 elective abortions) compared to 19 (21%) of 89 INH-unexposed pregnancies (including 3 elective abortions). A composite adverse pregnancy outcome was observed in 13 (33%) of 39 INH-exposed versus 16 (18%) of 89 INH-unexposed. The odds ratio, adjusted for covariates at or proximal to pregnancy outcome, of non-live birth for INH-exposed versus INH-unexposed was 1.87 (95% CI 0.75-4.69) and of adverse pregnancy outcome was 1.73 (95% CI 0.67-4.50; Table).**Conclusion:** INH exposure starting in the first trimester was associated with increased adverse pregnancy outcomes in this trial. While none of the associations reached statistical significance in analyses adjusted for covariates at or proximal to delivery, this finding supports other recent data from the TEMPRANO and P1078 trials.

179 **PrEP USE DURING ACUTE HIV INFECTION IN A COMMUNITY SETTING COMPROMISES HIV DIAGNOSIS**

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$^1$Weill Cornell Medicine, New York, NY, USA, $^2$Umbrella University of Science and Technology, Mbarara, Uganda, $^3$Cornell University, Ithaca, NY, USA, $^4$University of California San Diego, San Diego, CA, USA, $^5$Immunec Suppression Syndrome Clinic, Mbarara Regional Referral Hospital, Mbarara, Uganda, $^6$University of California San Francisco, San Francisco, CA, USA

**Background:** HIV counseling and testing is an essential component of controlling the HIV/AIDS epidemic. However, uptake of HIV testing is sub-optimal in sub-Saharan Africa. For example, in rural Uganda, only 39% of sexually active adults have received an HIV test within the last 12 months. In Uganda, and throughout sub-Saharan Africa, traditional healers are ubiquitous, informal providers who are frequently preferred for healthcare services, and are more accessible than biomedical clinics. We hypothesized that involving traditional healers in HIV testing may increase uptake in a rural, endemic region.

**Methods:** We conducted a cluster randomized trial of an HIV testing program to determine the effectiveness of traditional healers delivering HIV testing to directly to adults receiving care at their practices. The unit of randomization in this trial was the traditional healer site as a cluster. Outcomes were assessed at the level of the individual client of participating healers. Traditional healers and their clients in Mbarara Township were randomized to an intervention group or standard of care (control). Intervention arm healers delivered point-of-care oral swab HIV tests to sexually active adult clients who reported no HIV testing within the prior 12 months. Control arm healers provided referral for HIV testing at nearby medical clinics. Primary outcome for this trial was individual clients receiving an HIV test within 90 days of their visit. Secondary outcomes were new HIV diagnosis and linkage to care for those newly diagnosed.

**Results:** Between August 2019 and February 2020, 17 traditional healers were randomized (9 intervention, 8 control) and 500 clients of unknown HIV status significantly increased the rate of HIV testing, diagnosis, and linkage to care in rural Uganda. This novel, community-based approach holds promise for increasing uptake of HIV testing in Sub-Saharan Africa.

180 **A CLUSTER-RANDOMIZED TRIAL OF TRADITIONAL HEALERS DELIVERING HIV TESTING IN UGANDA**

Radhika Sundararajan$^1$, Juliet Mwangwa-Amumpaire$^2$, Matthew Pontiello$^3$, Myung Hee Lee$^6$, Stefanie Strathdee$^6$, Winnie Muyindike$^2$, Denis Nansera$^2$, Rachel King$^2$, Daniel Fitzgerald$^3$

$^1$Weill Cornell Medicine, New York, NY, USA, $^2$Umbrella University of Science and Technology, Mbarara, Uganda, $^3$Cornell University, Ithaca, NY, USA, $^4$University of California San Diego, San Diego, CA, USA, $^5$Immunec Suppression Syndrome Clinic, Mbarara Regional Referral Hospital, Mbarara, Uganda, $^6$University of California San Francisco, San Francisco, CA, USA

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**Table:** Clinical and diagnostic test results from 6 Thai MSM who started PrEP during acute HIV infection. x = generation, y = antibody test,

<table>
<thead>
<tr>
<th>Participant</th>
<th>Pre-PrEP VL (copies/mL)</th>
<th># days on PrEP</th>
<th>Pre-ART VL (copies/mL)</th>
<th>Pre-ART CD4 (cells/mm$^3$)</th>
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**Conclusion:** Delivery of point-of-care HIV tests by traditional healers to adults receiving care within the last 12 months of unknown HIV status significantly increased the rate of HIV testing, diagnosis, and linkage to care in rural Uganda. This novel, community-based approach holds promise for increasing uptake of HIV testing in Sub-Saharan Africa.
HIV SELF-TESTING TO OPTIMIZE FACILITY TESTING: A CLUSTER-RANDOMIZED TRIAL IN MALAWI

Kathryn Dovel1, Sundeep Gupta1, Christian Stillson2, Misheck Mphande1, Kelvin Balakasi1, Isabella Robson1, Chi-Hong Tseng1, Alemayehu Amberbir1, Leslie Berman1, Shaukat Khan Khan1, Joep J. Van Oosterhout3, Naoko Doi1, Brooke Nichols1

1University of California Los Angeles, Los Angeles, CA, USA, 2Clinton Health Access Initiative, Boston, MA, USA, 3Partners in Hope, Lilongwe, Malawi, 4Boston University, Boston, MA, USA

Background: High coverage of HIV testing among outpatients in generalized epidemics is critical for sustained epidemic control; however, severe human resource and infrastructure constraints have made this impractical in LMICs.

HIV self-testing (HVST) distributed at outpatient departments is a promising resource and infrastructure constraints have made this impractical in LMICs.

Methods: A cluster randomized implementation trial was conducted at 9 facilities in Malawi between February–April, 2020. Facilities were randomized 1:1:1: PITC (provider-initiated one-on-one testing); Passive HVST (group Oraquick® demonstration and outpatient/guardian-initiated HVST distribution); and Active HVST (group Oraquick® demonstration, provider-initiated one-on-one HVST distribution). All activities took place prior to routine consultations and were implemented by cadres with no more than a secondary school certificate. The primary outcome was self-reported same-day HIV-testing among eligible (never tested positive and never tested or last tested > 12 months ago) adults, using a difference-in-differences analysis (2-weeks pre and 3-weeks post intervention). Exit surveys were conducted with a systematically sampled subset of adult outpatients and guardians. Healthcare worker time was observed by study staff.

Results: 3,182 adults were enrolled (1851 outpatients, 1331 guardians): 33% were male and 38% were eligible for HIV testing (Table). HIV testing among eligible individuals was low across arms, with highest coverage in Active HVST post-intervention (26%). There was a 13 percentage-point increase (95% CI: 4.2–22.8%) in testing between Active HVST compared to PITC post-intervention, but no significant difference between Passive HVST and PITC. Increased coverage was mainly among adult guardians. Active HVST had the highest positivity (8/101, 8%, 95% CI: 2.3–13.2%), with 7/8 (88%, 95% CI: 47–99%) reporting same-day ART initiation. Total provider time required per test completed was estimated to increase 8% post-intervention in PITC, and decrease by 36% and 53% in Passive and Active HVST, respectively.

Conclusion: Facility HVST in outpatient departments implemented by lower-cadre cadres can increase testing coverage and both testing and health worker efficiency within routine testing settings.
POINT-OF-CARE URINE TENOFOVIR VERSUS SELF-REPORTED ART ADHERENCE IN ROUTINE HIV CARE

Tamsin K. Phillips1, Yolanda Gomba1, David Huang1, Landon Myer3
1University of Cape Town, Cape Town, South Africa, 2Western Cape Provincial Department of Health, Cape Town, South Africa

Background: The Abbott point-of-care (POC) urine lateral flow assay for tenofovir (TFV) was developed and validated on stored specimens from patients on pre-exposure prophylaxis. We compared the POC urine TFV test to self-reported ART adherence among patients in routine HIV care in Cape Town, South Africa.

Methods: Adults living with HIV, on a TFV-containing regimen and having a routine viral load (VL) test at a large ART clinic were enrolled in a cross-sectional study. Interviews collected demographic information and self-reported missed ART doses in the past 7 days. A urine sample was collected and immediately tested using the POC urine TFV test. VL results from the day of visit were abstracted from medical records.

Results: 314 patients were enrolled Feb-Nov 2020 (mean age 39 years, 79% female, 61% >5 years on ART). Most patients were on Efavirenz+FTC/TDF+TFV. Only 20 patients (6%) had no urine TFV detected. VL was <50 and <1000 copies/mL in 259 (82%) and 293 (93%) patients, respectively. Among 55 patients with VL ≥50, 13 had no urine TFV detected (sensitivity 24%, 95% CI 13-37). Among 259 patients with VL <50, 252 had urine TFV detected (specificity 97%, 95% CI 95-99). Using a threshold of 1000 copies/mL, there was higher sensitivity (n=21 with VL ≥1000: sensitivity 52%, 95% CI 30-74), but similar specificity (n=293 with VL <1000: specificity 97%, 95% CI 94-99). Results were independent of patient sex and weight. Self-reported adherence was high. Only 11% of patients (33/314) reported ≥2 missed ART doses in the past 7 days; 5% (15/314) reported missing >2 doses. Compared to self-report, the POC urine TFV test had a higher sensitivity, specificity and area under the receiver operating curve (ROC) to predict VL ≥50 and VL ≥1000, although confidence intervals were overlapping (Figure). Among 21 patients with VL ≥1000 copies/mL, 10 (48%) had urine TFV detected and 16 (76%) reported no missed doses in the past 7 days.

Conclusion: In this largely adherent cohort, TFV detected on the POC urine test identified almost all suppressed patients and was a better predictor of VL than self-report. Although sensitivity was low, this simple POC test could prompt adherence discussions with patients with no TFV detected. There may also be value in combining this objective adherence measure with VL test results to flag patients with raised VL in the presence of ART. Further research is needed to understand the practical applications of this POC test for patient care, particularly in non-adherent populations.

RAPID VS SAME-DAY TREATMENT INITIATION FOR PATIENTS WITH TB SYMPTOMS AT HIV DIAGNOSIS

Nancy Dorvil1, Cynthia Riviire1, Patrice Severe1, Jessy Devieux1, Kerylyne Lavoile1, Stephanie Bousleiman1, Etienne Cremieux1, Emelyne Dumont1, Alexandra Apollon1, Benedict Charles1, Giovani Saintyl1, Mikerylne Faustin1, Jean W. Pape1, Serena Koenig1
1GHESKIO, Port-au-Prince, Haiti, 2University of California Davis, Davis, CA, USA, 3Florida International University, Miami, FL, USA, 4Harvard Medical School, Boston, MA, USA, 5Brigham and Women's Hospital, Boston, MA, USA

Background: Delays in ART initiation for TB testing are associated with high rates of loss to follow-up. There are limited data on outcomes with same-day testing and treatment for patients with TB symptoms at HIV diagnosis.

Methods: We conducted a randomized trial comparing same-day and rapid (7 days) TB testing and treatment initiation among adult patients with TB symptoms at HIV diagnosis at GHESKIO in Haiti. The same-day group received Xpert Ultra results and initiated either TB medication or ART on the day of HIV diagnosis. The rapid group received Ultra results within the first week and started ART on Day 7 if not diagnosed with TB. Dolutegravir (DTG) replaced efavirenz (EFV) as the first-line anchor drug in December 2018. The primary outcome was 48-week HIV-1 RNA <200 copies/mL.

Results: Between November 2017 and December 2019, 500 participants were randomized to rapid (n=250) or same-day treatment (n=250) (Table 1). 234 (46.8%) were female, median age was 37 (IQR: 30, 45), and median CD4 count was 278 (134, 421). In the rapid group, 40/41 (97.6%) participants diagnosed with TB started TB drugs; 244 (97.6%) started ART. In the same-day group, 45/45 (100%) diagnosed with TB started TB drugs; 250 (99.6%) started ART. There were no statistically significant differences in 48-week outcomes between groups. In the rapid group, 224/250 (89.6%) were retained in care, and of these, 171 (76.3%) had HIV-1 RNA <200 copies/mL. In the same-day group, 219/250 (87.6%) were retained in care, and of these, 155 (70.8%) had HIV-1 RNA <200 copies/mL. The primary outcome was achieved by 171/250 (68.4%) in the rapid group and 155/250 (62.0%) in the same-day group (p=0.133). Outcomes were superior among participants who initiated ART with DTG instead of EFV, with HIV-1 RNA <200 copies/mL in 82.4% and 87.6% in the rapid and same-day groups, respectively (p=0.001) among those receiving viral load testing, and 75.3% vs. 60.4% among those randomized (p<0.001).

Conclusion: Among patients with TB symptoms at HIV diagnosis, both rapid and same-day treatment are associated with near-universal initiation of TB treatment and ART, with no significant difference in 48-week outcomes. Viral suppression rates were lower than anticipated, which we attribute to high rates of transmitted EFV resistance, political instability with a national lockdown, and the SARS-CoV-2 outbreak in Haiti during the study period. Viral suppression rates are superior with DTG, supporting the rapid transition from EFV to DTG-based ART.
COMMUNITY DISTRIBUTION OF ART DURING CIVIL UNREST AND COVID-19 IN HAITI

Patrice Joseph1, Hoi Ching Cheung3, Neil Sequiera1, Jean Edouard Mathon1, Marc-Antoine Jean-Juste, Young Macius2, Rody Secours2, Colette Guiteau3, Karine Sever4, Nancy Dorvil1, Eli Maxime Francois5, Adias Marcelin5, Rose-Irene Verdier4, Marie Marchelle Deschamps7, Jean W. Pape1

1GHESKIO, Port-au-Prince, Haiti; 2Analysis Group, Inc, Boston, MA, USA

Background: Challenges to retain patients with HIV in Haiti were worsened by civil unrest and the COVID-19 pandemic. To support patient retention, GHESKIO, one of the largest HIV care centers in the Caribbean, set up 11 community distribution points (CDPs) for antiretroviral therapy (ART) pickup and viral load testing at satellite sites in Port-au-Prince neighborhoods, and offered home delivery to patients.

Methods: The choice to pick up ART at CDPs was offered to all patients by 5/2019. Nurses at CDPs referred patients to GHESKIO clinics if they were symptomatic or due for physician visit. Data on all ART pickups in 5/1/2019-10/23/2020 from GHESKIO’s electronic health records were described.

Multivariable logistic regressions were used to identify patient characteristics associated with having ≥1 non-clinic visit (i.e. at CDP or home).

Results: 16,234 patients completed ≥1 drug pickup visits during the study period (41.2% male; mean±SD age 41.8±13.3 years; 14% newly initiated ART since 5/2019; 6.0±4.1 years since ART enrollment as of 5/2019 among previously enrolled patients). 39.3% of patients had ≥1 non-clinic pickup (31.8% had ≥1 CDP visit, 12.7% had ≥1 home visit). Patients attended 77,514 visits (4.8±2.2 visits per patient); proportions of visits at CDPs and home increased to 27.5% and 4.7%, respectively. Of patients with visits since 3/2020, 2,824 (18.6%) patients relied solely on non-clinic ART pickups (13.7% only at CDPs; 3.3% only at home). Regression suggests male sex, higher education, higher income, age <18 years, longer time since ART initiation, and non-single civil status were associated with having ≥1 non-clinic visit. Patients living in Carrefour, a neighborhood blocked from GHESKIO clinics during civil unrest, were more likely to have ≥1 non-clinic visit than patients from other neighborhoods.

Conclusion: Community distribution of ART builds resilience in health systems and supports continuity of care when access to clinic is limited. These services may be especially preferred by younger patients with longer time since ART initiation, higher income and education, and living in areas with limited access to medical clinics.

Logistic Regression Results

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<th>P-value</th>
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<td>1.70</td>
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<td>Other</td>
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<td>(0.17, 0.21)</td>
<td>&lt;0.001*</td>
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</table>

RESILIENCE OF HIV ACTIVITIES DURING COVID-19 PANDEMIC AT HEALTH FACILITIES IN AFRICA

Tiffany G. Harris1, Edward G. Jasi1, Carlos G. Laudari, Bonaparte Nijirazana1, Hermann Brou1, Faustin Malele2, Ruben G. Sahabo3, Zenebe Melaku, Mark Hawken1, Mirriah Vitalé, Florence Bayoa4, Priska Kasonde5, Wafa M. El-Sadri1

1ICAP at Columbia University, New York, NY, USA; 2ICAP at Columbia University, Luanda, Angola; 3ICAP at Columbia University, Addis Ababa, Ethiopia; 4ICAP at Columbia University, Kisumu, Kenya; 5ICAP at Columbia University, Maputo City, Mozambique

Background: The COVID-19 pandemic has impacted healthcare access due to travel restrictions, fear of exposure at health facilities (HF), changes in national policies and redirection of resources. We aimed to examine the impact that COVID-19 had on specific HIV activities including HIV testing, antiretroviral therapy (ART) initiation and viral load (VL) testing and suppression (VLS) at President’s Emergency Plan for AIDS Relief (PEPFAR)-supported HF in 11 African countries.

Methods: Retrospective routine data collected quarterly (Q) [Q1:October-December 2019; Q2: January-March 2020; Q3:April-June 2020; Q4:July-September 2020] from 1059 (ICAP-supported HF in Angola (HF=17), Burundi (HF=88), Cameroon (HF=73), Cote d’Ivore (HF=145), the Democratic Republic of Congo (HF=199), Eswatini (HF=42), Ethiopia (HF=31), Kenya (HF=1), Mozambique (HF=59), South Sudan (HF=20) and Zambia (HF=384) were analyzed to determine quarterly trends along the HIV testing and treatment cascade.

Results: Overall, there was a 3.3% decrease in the number HIV tested from Q2 (572,845) to Q3 (553,780) (Figure 1). This change varied by country ranging from a 57% decrease in Kenya (5,460 to 2,364) to a 104% increase in Cameroon (45,940 to 93,735). The number testing HIV-positive in all countries declined by 5.0% from Q2 (22,662) to Q3 (21,553) with little change in yield (4.0% vs. 3.9%). In Q4 the number HIV tested increased by 10.6% (to 612,646) from Q3, and the number testing HIV-positive in all countries declined by 9.0% (23,457) with little change in yield (4.0% vs. 3.9%). (45,940 to 93,735). The number testing HIV-positive in all countries declined by 5.0% from Q2 (22,662) to Q3 (21,553) with little change in yield (4.0% vs. 3.9%).

Conclusion: In this large study, with the of COVID-19 pandemic acceleration from Q2 to Q3, the number HIV tested decreased along with declines in number of HIV+ persons identified and new ART initiations. However, rebound was brisk as the pandemic progressed (Q4), demonstrating remarkable HIV program resilience. The number on ART, VL testing and VLS continued to increase throughout the period. This may have been, in part, due to recent expansions of non-HF-based differentiated service delivery models that include more diverse groups.
HOST-PATHOGEN INTERACTIONS OF HIGHLY PATHOGENIC CORONAVIRUSES REVEAL DRUG TARGETS

David E. Gordon, Mehdi Bouhaddou, Veronica V. Rezelj, Kris M. White, Matthew J. O’Meara, Guangdiyun M. Jang, Jeffrey Z. Guo, Joseph M. Hiatt, Kirsten Obernier, Pedro Beltrao, Marco Vignuzzi, Adolfo Garcia-Sastre, Kevan Shokat, Brian K. Shoichet, Nevan J. Krogan, for the QB3 Coronavirus Research Group

Background: The novel coronavirus SARS-CoV-2, the causative agent of COVID-19, has caused worldwide social and economic disruption. Initial efforts to treat SARS-CoV-2 were hampered by limited knowledge of the molecular details of SARS-CoV-2 infection. To identify molecular targets for SARS-CoV-2 therapeutics, we mapped the host-pathogen protein interactions of SARS-CoV-2, and investigated host dependency pathways that are required for SARS-CoV-2 infection using drug, knockdown and knockout screens. Concerns regarding the mutagenic potential of SARS-CoV-2 also led us to inquire whether a conserved set of human host factors may be required for infection by all highly pathogenic coronaviruses, thus representing pan-coronavirus drug targets. Therefore, we also mapped the host protein interactions of SARS-CoV-1 and MERS-CoV.

Methods: We cloned, tagged and expressed proteins encoded by SARS-CoV-2, SARS-CoV-1, and MERS-CoV in HEK-293T cells, which are permissive to infection by all three viruses. Cells expressing individual proteins were harvested, affinity purifications performed in 96-well format, and protein mass spectrometry was utilized to identify physical interaction partners of each viral protein. Drug treatments, RNAi knockdowns and CRISPR/Cas9 knockouts were tested for SARS-CoV-2 viral phenotypes in Vero, Caco2 or A549-ACE2 cells.

Results: We report 389 high-confidence interactors of SARS-CoV-2, 366 interactions for SARS-CoV-1, and 296 interactions for MERS-CoV. Among the SARS-CoV-2 interactors, we identified at least 66 druggable human proteins or host factors, and screening these molecules targeting these pathways using multiple viral assays have identified at least four sets of pharmacological agents that demonstrate antiviral activity against SARS-CoV-2. Comparison of the host-pathogen interactomes of SARS-CoV-2 with the other highly pathogenic coronaviruses SARS-CoV-1 and MERS highlights shared host interactions which may represent pan-coronavirus drug targets.

Conclusion: We successfully utilized systematic protein interaction mapping to identify drug targets for SARS-CoV-2, leading to several Covid-19 clinical studies investigating the efficacy of drugs perturbing these pathways. Furthermore, comparative proteomics of the related coronaviruses SARS-CoV-1 and MERS-CoV identified shared host interactions which may represent pan-coronavirus drug targets. For a full list of contributing authors see: Gordon, D. E. et al. Nature 583, 459–468 (2020); Gordon, D. E. et al. Science 370 (2020).

SYSTEMATIC ANALYSIS OF SARS-CoV-2 INFECTION OF AN ACE2-NEGATIVE HUMAN AIRWAY CELL


Background: Established in vitro models for SARS-CoV-2 infection are limited and include cell lines of non-human origin and therefore engineered to overexpress ACE2, the cognate host cell receptor. Although Calu-3, a human lung cell line which endogenously expresses ACE2, supports SARS-CoV-2 replication, they are significantly less permissive to infection than other models. Furthermore, ACE2 expression in the respiratory tract is low and emerging evidence suggests the utilization of alternative host cell receptors and attachment factors may compensate for low ACE2 expression levels in the lung. We identified human HS22 lung adenocarcinoma cells as naturally permissive to SARS-CoV-2 infection despite complete absence of ACE2.

Methods: A panel of 10 cell lines, with variable expression levels of ACE2 and TMPRSS2 were infected with SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Viral replication was monitored through assessment of cell-associated and cell-free viral RNA (vRNA) by QRT-PCR as well as FACS and in situ hybridization. Effect of blocking S protein by neutralizing antibodies and an ACE2-Fc decoy peptide, ACE2 blocking by a specific antibody, and ACE2 knockout by CRISPR on SARS-CoV-2 replication was determined by Q-RT-PCR for vRNAs. Various viral entry inhibitors were used to pathway of SARS-CoV-2 entry in HS22 cells. RNA sequencing and proteomics was used to study the cell and innate immune responses in infected HS22 cells. siRNA-mediated knockdown was utilized to further characterize the pathway of immune sensing.

Results: Infection of HS22 cells required the SARS-CoV-2 spike protein, though in contrast to ACE2-dependent models, spike alone was not sufficient for HS22 infection. Temporally resolved transcriptomic and proteomic profiling revealed alterations in cell cycle and the antiviral host cell response, including MDA5-dependent activation of type-1 interferon signaling. Focused chemical screens point to important roles for chlorinate-mediated endocytosis and endosomal cathepsins in SARS-CoV-2 infection of HS22 cells.

Conclusion: These findings imply the utilization of an alternative SARS-CoV-2 host cell receptor which may impact tropism of SARS-CoV-2 and consequently human disease pathogenesis.

SARS-CoV-2 SPIKE PROTEIN INDUCES MONOCYTE APOPTOSIS AND INTERLEUKIN-8 PRODUCTION

Aswath Padmanabhan Chandrasekar, Mark Maynes, Sekar Natesampillai, F.N.U. Shweta, Andrew D. Badley, Nathan W. Cummins

Background: In the setting of SARS-CoV-2 infection and COVID-19 illness, a subset of symptomatic patients has been reported to experience severe leukopenia. Viral proteins have been described to have the capacity to induce cell death in peripheral blood cells in infections such as HIV. Given the expression of the cognate receptor, ACE-2, on the surface of Peripheral blood mononuclear cells (PBMCs), we hypothesized that SARS–CoV-2 may induce leukopenia via spike protein ligand–receptor interaction.

Methods: PBMCs were isolated from the fresh blood of normal donors and were treated with 1ug/ml of recombinant spike protein, and analyzed for cell death via the Incucyte Live/Cell imaging system. To measure subset specific cell death, PBMCs treated with recombinant spike protein for 48 hours were analyzed by flow cytometry for the expression of cell specific surface receptors and concomitant active caspase 3 expression. Culture supernatant was analyzed by multiplex cytokine analysis to evaluate the presence of pro-inflammatory cytokines. Similar assays were carried out in the presence of a spike- binding domain-antagonistic antibody in order to determine the specific role of spike-ACE2 interaction in causing cell death. Finally, cells from COVID positive patients were analyzed to determine if similar results were observable in vivo.

Results: The treatment of PBMCs with recombinant SARS-CoV-2 spike resulted in significant cell death over time in 2 out of three donors tested (p<0.05) by Incucyte live imaging analysis. When analyzed for subset specific cell death, a significant increase in cell death (p<0.01), as measured by Caspase 3, was observed in CD14+ CD3- cells, correlating with the monocyte population. Supernatants from these cultures demonstrated markedly increased IL-8 production (p=0.0536). Cultures carried out in the presence of a spike antagonistic antibody abrogated the effects of spike protein, indicating a direct relationship between spike-ACE2 interaction and cell death in this sub-population. Similar flow cytometric analysis from 5 febrile patients with COVID-19 demonstrated significantly increased monocyte apoptosis (p<0.05), compared to CD3-lymphocytes from the same donors; whereas significantly increased monocyte apoptosis was not observed in 5 afebrile COVID-19 patients.

Conclusion: These results indicate that SARS-CoV-2 spike protein may induce apoptosis specifically in Monocytes, in an ACE2 dependent manner, in some but not all patients.
190 LUCIFERASE COMPLEMENTATION ASSAY FOR IDENTIFICATION OF SARS-CoV-2 3CLpro INHIBITORS
Jonathan Rawson1, Alice Duchon1, Olya Nikoiiitchik2, Vinay K. Pathak1, Wei-Shau Hu1
1National Cancer Institute, Frederick, MD, USA
Background: The 3C-like protease (3CLpro) of SARS-CoV-2 has been widely pursued as a target for COVID-19 anti-viral drug development because it is essential for viral replication and lacks significant homology to human proteases. However, drug development for 3CLpro has been hindered by a lack of cell-based reporter assays that can be performed in a BSL-2 setting. Current efforts to identify 3CLpro inhibitors largely rely upon in vitro screening, which fails to account for the cell permeability and cytotoxicity of compounds, or assays involving replication-competent virus, which must be performed in a BSL-3 facility and are not amenable to high-throughput screening.

Methods: To address these limitations, we explored the use of a cell-based luciferase complementation reporter to identify inhibitors of SARS-CoV-2 3CLpro in BSL-2 setting. We constructed lentiviral vectors that co-express 3CLpro and a split reporter in which two luciferase fragments were linked by a 3CLpro cleavage site. The 3CLpro-mediated cleavage of the reporter was expected to result in loss of complementation and low luciferase activity, whereas inhibition of 3CLpro was expected to result in significantly higher levels of luciferase activity.

Results: In the absence of inhibitors, we found that most of the luciferase reporter was cleaved by 3CLpro, resulting in low luciferase activity. However, inhibition of 3CLpro, either with the small molecule GC376 or an inactivating mutation (C40A), prevented cleavage and resulted in an ∼10-fold increase in luciferase reporter activity. We also found that our reporter assay can easily distinguish between cytotoxicity and true inhibition of 3CLpro. With this assay, we screened 31 additional small molecules for activity against SARS-CoV-2 3CLpro, including HIV protease inhibitors, HCV protease inhibitors, and various other compounds that have been reported to inhibit 3CLpro. Of these, only four compounds exhibited significant activity against SARS-CoV-2 3CLpro in cells: boceprevir, Z-FA-FMK, calpain inhibitor XII, and GRL-0496.

Conclusions: We developed a novel luciferase complementation reporter assay for identification of SARS-CoV-2 3CLpro inhibitors in living cells. The assay is sensitive, rapid, easy to perform, and can readily differentiate cytotoxicity from 3CLpro inhibition, a powerful feature that should reduce false positives during screening. This assay should greatly facilitate efforts to identify more potent inhibitors of SARS-CoV-2 3CLpro.

191 EXCESS OF SARS-CoV-2 SUBGENOMIC RNAs IN CELLS AND FLUIDS DURING ACUTE INFECTION
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Background: SARS-CoV-2 is transcribed as genomic RNA (gRNA) and different subgenomic RNAs (sgRNA), allowing variation in viral gene expression. However, the extent and clinical significance of subgenomic transcription remain unknown. We hypothesized that SARS-CoV-2 RNA levels would vary between genome regions and between patients, tissues, and sample types but remains unclear. We sought to measure the subgenomic RNA levels in nasopharyngeal (NP) swabs from 3 SARS-CoV-2 infected patients. The results suggest a greater excess of sgRNAs in the cell-free fluids.

Methods: To address these limitations, we explored the use of a cell-based luciferase complementation reporter to identify inhibitors of SARS-CoV-2 3CLpro in BSL-2 setting. We constructed lentiviral vectors that co-express 3CLpro and a split reporter in which two luciferase fragments were linked by a 3CLpro cleavage site. The 3CLpro-mediated cleavage of the reporter was expected to result in loss of complementation and low luciferase activity, whereas inhibition of 3CLpro was expected to result in significantly higher levels of luciferase activity.

Results: In the absence of inhibitors, we found that most of the luciferase reporter was cleaved by 3CLpro, resulting in low luciferase activity. However, inhibition of 3CLpro, either with the small molecule GC376 or an inactivating mutation (C40A), prevented cleavage and resulted in an ∼10-fold increase in luciferase reporter activity. We also found that our reporter assay can easily distinguish between cytotoxicity and true inhibition of 3CLpro. With this assay, we screened 31 additional small molecules for activity against SARS-CoV-2 3CLpro, including HIV protease inhibitors, HCV protease inhibitors, and various other compounds that have been reported to inhibit 3CLpro. Of these, only four compounds exhibited significant activity against SARS-CoV-2 3CLpro in cells: boceprevir, Z-FA-FMK, calpain inhibitor XII, and GRL-0496.

Conclusions: We developed a novel luciferase complementation reporter assay for identification of SARS-CoV-2 3CLpro inhibitors in living cells. The assay is sensitive, rapid, easy to perform, and can readily differentiate cytotoxicity from 3CLpro inhibition, a powerful feature that should reduce false positives during screening. This assay should greatly facilitate efforts to identify more potent inhibitors of SARS-CoV-2 3CLpro.

192 IDENTIFICATION OF A NEW LENTIVIRUS RESTRICTION COUNTERACTED BY VPR
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Background: Lentiviruses encode non-structural accessory proteins that function to counteract host restriction factors. The lentivirus protein Vpr is known to block G2 to M transition of the cell cycle and degrades various host DNA repair proteins, including Uracil-N-Glycosylases: UNG2 and SMUG1. The reason why Vpr induces these cellular changes in the infected cell is unknown.

Methods: We explored this question by direct injection of primary resting and activated CD4 T cells with a CRS-tropic replication-competent GFP reporter virus. We measured GFP expression by flow cytometry and virus expression by ELISA. We also performed bulk and single-cell RNAseq of sorted GFP+ cells to measure host mRNAs and virus mRNAs. Additionally, we used a Uracl-3qPCR to quantitatively localize provirus uracil versus thymidine incorporation. Lastly, we stimulated CellTrace labelled infected resting cells with and without Vpr and measured cellular proliferation in the constant presence of ART.

Results: We detected resting GFP+ cells 3 to 4 days after infection.

Transcriptome analysis of resting GFP+ versus activated GFP+ revealed a pathway for dNTP production in resting CD4 T cells that deoxyuracil is present instead of thymidine. We confirmed provirus uracil incorporation using the Uracl-3qPCR assay. Stimulation of infected resting CD4 T cells showed infected cells are prevented from dividing by Vpr. Lastly, we found replication-competent virus was only produced from the initially infected parent cells instead of the divided daughter cells.

Conclusion: We conclude HIV can directly infect primary resting CD4 T cells. HIV-infected resting CD4 T cells incorporate uracil instead of thymidine. After T cell stimulation, Vpr prevents infected cells from dividing because it counteracts an innate lentivirus restriction mechanism against the integrated provirus through UNG2 and SMUG1 recognition of the incorporated uracil.

193 MULTIPLY INFECTED ACH2 CELLS ARE RESPONSIBLE FOR THE MAJORITY OF ACH2 HIV PRODUCTION
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Background: ACH2 cells, A3.01 cells, singly infected by replication competent LAV, have been used as a model of latent infection. They continue to be studied because of their availability, ease of use and known proviral integration and isolation site.

Methods: TNF stimulated and unstimulated ACH2 cells were stained with fluorescein-labeled anti-envelope mAb (PG9 and VRC01) and sorted into envelope bright, dim, and null populations. Intracellular HIV RNA was measured both using primers and probes specific for D1 unspliced, D1A4b/D1A5 and 2KB env transcripts and a split reporter in which two luciferase fragments were linked by a 3CLpro cleavage site. The 3CLpro-mediated cleavage of the reporter was expected to result in low luciferase activity. However, we hypothesized that SARS-CoV-2 RNA levels would vary between genome regions and between patients, tissues, and sample types (cells or fluids).

Methods: We designed and validated 7 novel RT-ddPCR assays that target the 5' and 3' untranslated regions (UTR), non-genomic structural genes found only in full length gRNA (Main Proteinase (NSP5) and RNA dependent RNA polymerase (RdRpo)), and 3' structural genes (spike (S), membrane (M), and nucleocapsid (N)) that are also contained in different sgRNAs. Assay efficiencies were measured on standards derived from plasmids and virion stock supernatants. Levels of all 7 RNA regions were measured in nucleic acid extracted by the Abbott m000 platform from nasopharyngeal (NP) swabs from 3 SARS-CoV-2-infected individuals, and in cells and supernatant from NP, oropharynx (OP), and saliva isolated from 3 additional individuals.

Results: In all samples, levels of 3 targets (M, N, and 3'UTR) tended to be higher than 5 targets (5'UTR, NSP5, and RdRpo), suggesting an excess of 3' sgRNAs (3'UTR/5'UTR=2.4-6.2 and nucleocapsid/RdRpo=1.7-3.5 for NP samples; n=6, p=0.03). All SARS-CoV-2 RNAs were detected in both cells and supernatant from NP, OP, and saliva, but tended to be higher in the NP than OP. In saliva but not NP or OP, levels of gRNA per sample were consistently higher in the cells compared to supernatant per cell (sup/cell=2.7-4.8). Surprisingly, the excess of 3' over 5' viral RNAs was even greater in the supernatant per cell (3'UTR/S'UTR=1.5-6.2) from NP, OP, and saliva (p=0.016 across all), suggesting a greater excess of sgRNAs in the cell-free fluids.

Conclusions: The higher levels of 3' targets suggest an excess of sgRNA in all samples. Assays that target 3' regions found in sgRNAs (N, 3'UTR) may be more sensitive for detecting SARS-CoV-2, but may not indicate infectious virus. The greater excess of 3' transcripts in cell-free fluids suggests that sgRNAs are released from cells and/or persist to a greater degree than gRNAs. Future studies should investigate how levels of sgRNA change over the course of infection in cells and cell-free fluids, and whether sgRNA levels correlate with measures of disease transmission or severity.
population and 14-45% in the Env bright population prior to and through 9h of TNF stimulation. By sorting unstimulated ACH2 cells into Env bright and Env null population and then maintaining the Env null population in R10 with 1μM Raltegravir it was possible to establish stable populations of cells that were 1) Env negative (<5% P24 positive) and 2) Env positive (>90% P24 positive). Doubling time of Env negative and Env positive cells were similar. The relative proportion of proviral DNA/cell determined by ratio of gag DNA:albumin DNA was 7 fold higher in Env bright than Env negative cells. Production of p24 by Env negative and bright cells was 0.026 and 1.1/fg/h/cell in unstimulated ACH2 and 1.7 and 59 fg/h/cell in TNF stimulated cells, respectively.

Conclusion: These data show that even low pass ACH2 contain a significant number of HIV-superinfected cells. These cells produce far more multiply spliced HIV RNA and far higher amounts of virus than cells which contain only 1 proviral copy and are responsible for the majority of HIV produced by bulk ACH2 cells.

194 miRNA'S PLASMA PROFILE ANTICIPATES LOSS OF VIROLOGICAL CONTROL IN ELITE CONTROLLERS

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Background: Elite Controllers (EC) are an exceptional group of people living with HIV (PLWH) that maintain undetectable plasma HIV-1 viral loads below the detection limit while not being on antiretroviral therapy (ART). However, ECs represent a heterogeneous population in terms of virological, immunological and clinical outcomes, and around 25% of them lose virological control overtime. Thus, the aim of this study was to analyze what factors at a gene transcription level, such as miRNAs, that may lead to a loss of spontaneous viral control in PLWH-EC.

Methods: Plasma samples of 18 subjects from the Spanish HIV HGM BioBank belonging to the AIDS Research Network and with data in the RIS cohort of HIV Controllers Study Group (ECRIS) were included in the study. A total of 12 ECs who experienced a loss of spontaneous virological HIV-1 control (2 measurements of VL above the detection limit in 12 months) were classified as transient controllers (TC), and another group of 6 ECs who persistently maintained virological control during the same follow-up period were called persistent controllers (PC). miRNA expression profiles were obtained using TaqMan™ Advanced miRNA Human Serum/Plasma Cards.

Results: TC individuals showed an up-regulation of plasma miRNA profile before the loss of virological control when compared to PC. From the 23 miRNA statistically significant differentiated between groups (P<0.05), the most highly expressed miRNAs (fold change > 4.0) were hsa-miR-27a-3p, hsa-miR-376a-3p and hsa-miR-199a-3p, which exhibited 4.8-, 4.2- and 4.0-fold increased expression, respectively. TC after loss the spontaneous virological control also showed an up-expressed miRNA profile when compared to PC except for the hsa-miR-457-5p, which showed a downregulated expression in TC group compared to PC. Of interest, the expression of has-miR-199a-3p was highly up-regulated in TCs in both conditions, before and after (fold change = 5) the loss of virological control. Interestingly, the majority of the most highly expressed miRNAs in TC before and after the loss of virological control are related with lipid and lipoprotein metabolism.

Conclusion: A miRNA expression pattern associated with the spontaneous loss of virological control and also virological progression of ECs may contribute to a better understanding of clinical outcomes in PLWH-EC. A specific miRNA pattern in PLWH-EC could be used as biomarkers for a quick screening of PLWH-EC.

195 IDENTIFICATION OF A NOVEL ANTI-HIV miRNA IN INTERLEUKIN-27–DIFFERENTIATED MACROPHAGES

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Background: Interleukin-27 (IL-27) is a pleiotropic cytokine that influences the innate and adaptive immune systems. It inhibits viral infection and regulates the expression of microRNAs (miRNAs). We recently reported that macrophages differentiated from monocytes in the presence of IL-27 and human AB serum resisted HIV-1 infection and showed significantly enhanced autophagy.

Methods: CD14+ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of healthy donors and cultured in presence of IL-27 and human AB serum to make AB- and control macrophages. Differentiated from monocytes in the presence of IL-27 and human AB serum resisted HIV-1 infection and showed significantly enhanced autophagy. To elucidate the factors associated with the IFN-independent mechanisms, miRAB40 was identified in differentiated macrophages and then infected with HIVΔ8Δ9 or a pseudotyped HIV (HIVΔucV), and anti-HIV effect was measured by a 24 antigen ELISA kit or luciferase assay. Induced interferon (IFN) in culture supernatants were quantified using subtype-specific ELISA kit for IFNs, β and α and autophagy assay was conducted using autophagy detection kit.

Results: The miRNA sequencing analysis revealed the expression of nearly 1000 known and 38 novel miRNAs. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis using probes specific to each novel miRNA confirmed that IL-27 differentially regulated the expression of 16 of the 38 miRNAs. Overexpression of the synthesized miRNA mimics revealed that miRAB40 had potent HIV-inhibiting and autophagy-inducing properties. ELISA demonstrated that miRAB40 induced IFN-α (26.5 + 7.2 pg/ml) and IFN-β (6.7 + 2.2 pg/ml) but not IFN-γ. In addition, miRAB40 partially suppressed both activities, although the same amount of B18R protein inhibited both activities induced by 100 pg/mL IFN-α & β, indicating that the two functions were induced via Type-I IFN-dependent and -independent pathways, respectively. To elucidate the factors associated with the IFN-independent mechanisms, miRAB40-target prediction analysis, real-time RT-PCR, and western blotting were conducted. However, we could not identify them.

Conclusion: We discovered a total of 38 novel microRNAs in AB mac and characterized that, dual-function miRNA, miRAB40, may provide novel insights.
into the miRNA-mediated regulation of autophagy induction and HIV inhibition via IFN expression.

**196** RAB11-FIP1-DEPENDENT AND INDEPENDENT HIV-1 ENVELOPE TRAFFICKING

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**Background:** The mechanism of incorporation of the envelope glycoprotein (Env) into HIV-1 particles is defined incompletely. Our lab has provided evidence supporting a model where Env, after synthesis and transport to the plasma membrane (PM) is endocytosed. This step is followed by trafficking through endosomal recycling compartment (ERC) to the sites of particle assembly. A cellular adaptor, Rab11-FIP1 (FIP1), plays a key role in Env incorporation for the NL4-3 and JR-FL isolates of HIV. This study further evaluated FIP1-dependent Env trafficking using NL4-3 and a panel of primary isolates of HIV-1.

**Methods:** HIV Env interactions with FIP1 were studied by immunofluorescence colocalization and proximity ligation assay (PLA). Env incorporation into virions and HIV replication kinetics were examined in T-cell lines.

**Results:** We demonstrated in situ interaction of Env and FIP1. We expressed a truncated FIP1 (FIP1C560-649) previously shown to inhibit membrane cargo exit from the ERC. A strong perinuclear PLA signal indicated that FIP1C560-649 entrapped Env in the ERC and prevented its trafficking back to the PM. The extreme C-terminal region of this construct FIP1C was critical for the interaction with HIV Env. We next examined whether FIP1C560-649 inhibited Env trafficking in primary HIV-1 isolates. Intriguingly, some HIV-1 strains escaped trapping by FIP1C560-649. To determine if the FIP1C-independent strains still required transit through the ERC, we utilized a catalytically inactive ubiquitin ligase RFFL that has been shown to halt recycling of cargo from the ERC to the PM. Expression of RFFL resulted in a collapsed, perinuclear ERC that retained Env derived from both FIP1C-dependent and FIP1C-independent isolates. We also targeted the C-terminal region of FIP1C in HIV cell lines using CRISPR/Cas9. Upon infection of knockout cells with NL4-3, a significant reduction in Env incorporation into virions was observed, and viral replication in culture was markedly diminished.

**Conclusion:** The present work indicates that trafficking through the ERC is a critical step for incorporation of Env into HIV-1 particles. PLA results suggest a direct interaction between Env and FIP1C. While all HIV-1 Env isolates require recycling from the ERC, some utilize FIP1C as an essential recycling adaptor, but others do not. We speculate that alternative recycling adaptors are involved in the incorporation of Env into particles for FIP1C-independent HIV-1 isolates.

**197** IRON CHELATOR PPyTe INHIBITS HIV-1 REPLICATION IN HUMANIZED MICE

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**Background:** Targeting host cell factors involved in HIV-1 replication holds promise for HIV-1 inhibition, as no resistance is expected. An emerging factor that others do not. We speculate that alternative recycling adaptors are involved in the incorporation of Env into particles for FIP1C-independent HIV-1 isolates.

**Methods:** We used a custom designed array of 42 known restriction factors to analyze the effect of PP1Te on regulation of these restriction factors of HIV-1. Further we knocked down the identified factors (p21 or CH25H) and then carried out HIV-1 infection in presence of PP1Te to confirm the role of these factors in PP1Te mediated HIV-1 replication. Finally, humanized mice were infected with HIV-1 and PP1Te was administered intraperitoneally to confirm in-vivo role of PP1Te in HIV-1 restriction.

**Results:** Significant upregulation of p21 and CH25H was observed in PP1Te treated T-cell lines. Knockdown of p21 or CH25H reversed HIV-1 inhibition by PP1Te. Treatment with non-related protein phosphatase-1 targeting compound I7E-03 inhibited HIV-1 in p21 or CH25H knockdown cells. PP1Te treatment significantly suppressed HIV-1 replication in PBMC and THP-1 derived macrophages infected with HIV-1(bBI) and HIV-1(B8-A). In vivo, intraperitoneal administration of PP1Te reduced HIV-1 replication as reflected by reduced levels of HIV-1 TAR RNA, env mRNA and gag mRNA in humanized B6C3F1-NSG mice.

**Conclusion:** Iron chelator PP1Te inhibits HIV-1 replication by inducing levels of p21 and CH25H. It showed efficacy in HIV-1 infected humanized mice, suggesting its utility in future antiretroviral therapy.

**198** QUANTITATIVE PHOSPHOPROTEOME ANALYSES OF PP1-TARGETING HIV-1 INHIBITOR I7E-03

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**Background:** Human immunodeficiency virus-1 (HIV-1) establishes long-lived stable reservoirs that can be reactivated when combination antiretroviral therapy (cART) is interrupted or drug resistance appears. This study further demonstrated that PP1Te is a promising antiretroviral agent, as no resistance is expected. An emerging factor that others do not. We speculate that alternative recycling adaptors are involved in the incorporation of Env into particles for FIP1C-independent HIV-1 isolates.

**Methods:** We performed label-free quantitative phosphoproteomics and proteomics analysis of non-infected cells and HIV-1 infected cells that were untreated or treated with I7E-03. The phosphorylation quantitative analysis was carried with no enrichment (NER), or with phosphopeptides enriched on Fe-NiA or TiO2. The phosphorylation of the selected candidate proteins was further validated using Western blot (WB) with okadaic acid (OA) as a positive control.

**Results:** I7E-03 significantly reprogrammed the cellular phosphoproteome but did not change host proteins expression levels. Biological pathway analysis showed that phosphorylation of proteins within TGF-β and PP1Ra/PP1R3a signaling pathways was primarily affected by I7E-03. TGF-β signaling pathway, I7E-03 significantly decreased phosphorylation levels of TGF-β2 on Ser-466 (~12.02-fold, p=1.37E-03). In PP1R3a/PP1R3b pathway, phosphorylation of Nucleophosmin 1 (NPM1) at Ser-125 was significantly downregulated (~20.15-fold, p=1.37E-09). The downregulation of NPM1 phosphorylation in the cells treated with I7E-03 was further confirmed by WB analysis.

**Conclusion:** We have identified TGF-β and PP1R3a/PP1R3b signaling pathway as being primarily affected by PP1-targeting HIV-1 transcription inhibitor I7E-03 using global quantitative phosphoproteomics and proteomics. TGF-β and NPM1 have been associated with HIV-1 transcription activation, and reported as PP1 partners or substrates. Therefore, targeting phosphorylation of host proteins such as TGF-β2 and NPM1 might serve as a novel approach to achieve HIV-1 transcription inhibition.

**199** A SORTING SIGNAL IN THE SIV Env TAIL IS SELECTED IN VIVO DURING PATHOGENIC INFECTION

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**Background:** The cytoplasmic tail of HIV and SIV Env contains a highly conserved tyr-dependent sorting motif, YxxØ (x= any a.a.; Ø= an a.a. with a bulky hydrophobic side chain) that mediates clathrin-dependent endocytosis and basolateral sorting of Env. Deletion of Gly and Tyr from this motif in SIVmac239 Env (a.a. 270-721), creating a virus termed AGO, resulted in a novel phenotype in pigtail macaques (PTM) characterized by preservation of gut pathology, and reduced disease phenotype in pigtail macaques (PTM) characterized by preservation of gut pathology, and reduced disease.
were used to assess the impact of R722G and ∆QTH Env mutations. PTM were inoculated with ∆GY containing R722G ± ∆QTH and animals followed for plasma viral RNA and viral evolution.

Results: In vitro, the ∆GY mutation decreased Env content in cells and on virions by 40%. An R722G mutation restored Env expression but had no effect on Env trafficking. In contrast, the ∆QTH mutation reconstituted potent Tyr-dependent signals for both endocytosis and basolateral sorting. In PTM, ∆GY containing both R722G and ∆QTH Env mutations was associated with high viral loads and were maintained throughout infection. Two animals that received ∆GY containing only R722G developed high viral loads and AIDS in association with further mutations: One animal developed a ∆QTH; the second acquired three point mutations creating a novel motif (IRL; Fig.1) that conferred basolateral sorting but not endocytosis. In vivo, when introduced onto a ∆GY background the IRL mutations were completely conserved and sufficient to maintain high viral loads in four PTM.

Conclusion: These findings reveal strong selection pressures on signals for Env endocytosis and basolateral sorting and reveal a previously unappreciated role for polarized cellular trafficking of Env during pathogenic lentivirus infection.

200 ADOPTIVE TRANSFER OF GAMMA DELTA T CELLS ENHANCES HIV INFECTION IN A HUMANIZED MOUSE

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Background: Human gamma-delta (γδ) T cells can mediate potent antiviral effects in an MHC-independent manner, making them attractive for immunotherapeutic application to treat chronic HIV infection. In vitro studies demonstrated the cytotoxic capacity of Vδ2 T cells against HIV infected targets. However, in vivo translational studies defining the antiviral properties of these cells are lacking. Here, we optimized a humanized mouse model to study the immunologic role, functional status, and immunotherapeutic potential of Vδ T cells in the setting of HIV infection.

Methods: Bone-marrow, Liver, Thymus (BLT) humanized mice (huMice) were generated by engrafting NSG mice with autologous hHSCs via retro-orbital injection, and with human lymphoid tissues (fetal thymus and liver) for hematopoeisis via kidney capsule transplant. Reconstitution, phenotype, and function of human immune cells were characterized by flow cytometry analysis. The immunotherapeutic potential of Vδ T cells was assessed by adoptive transfer of HIV-infected CD4 T cells and ex-vivo expanded allogeneic Vδ T cells into huMice. Blood samples from HIV-infected huMice were analyzed by flow cytometry and qRT-PCR to measure changes in the frequency and phenotype of human T cells and HIV viral load, respectively.

Results: We provide the first characterization of successfully reconstituted γδ T cell subsets in the peripheral blood and lymphoid tissue of BLT huMice, and we demonstrate an HIV-associated depletion of Vδ2 T cells and increase in Vδ1 T cells in the peripheral blood and lymphoid tissue of BLT huMice, and were maintained throughout infection. Two animals that received ∆GY containing only R722G developed high viral loads and AIDS in association with further mutations: One animal developed a ∆QTH; the second acquired three point mutations creating a novel motif (IRL; Fig.1) that conferred basolateral sorting but not endocytosis. In vivo, when introduced onto a ∆GY background the IRL mutations were completely conserved and sufficient to maintain high viral loads in four PTM.

Conclusion: These findings reveal strong selection pressures on signals for Env endocytosis and basolateral sorting and reveal a previously unappreciated role for polarized cellular trafficking of Env during pathogenic lentivirus infection.

201 EXPERIMENTAL MICROBIAL DYSBIOSIS ENHANCES RECTAL SIV ACQUISITION IN RHEUS MACAQUES

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Background: Alteration to the composition of the vaginal and rectal bacterial microbiomes is associated with localized inflammation and correlate with acquisition of some sexually transmitted pathogens such as HIV.

Methods: To directly assess the contribution of bacterial dysbiosis to rectal lentiviral acquisition, we induced dysbiosis in rhesus macaques prior to repeated, dose-intra-rectal challenge with SIVmac239X, utilizing the antibiotic vancomycin.

Results: Although no difference was noted in the number of challenges required for SIV acquisition, vancomycin administration led to significantly increased numbers of transmitted-founder variants detected upon SIV acquisition. Vancomycin-treated animals displayed decreased intestinal T-cell activation during acute SIV infection; however, these features did not distinguish between animals that acquired SIV at early versus late challenge. Early acquisition - irrespective of experimental dysbiosis -was associated with significantly reduced frequencies of rectal Th22 cells and IgA+ B-cells, with vancomycin-treated animals displaying a trend towards reduced Th22 frequencies. Th22 frequency correlated with the number of challenges required for infection. Significant differences in Ruminococcaceae, Gammaproteobacteria, and Prevotellaceae genera distinguished between early and late acquisition and were additionally perturbed in vancomycin-treated animals.

Conclusion: These findings experimentally demonstrate that intestinal dysbiosis contributes to alteration to gastrointestinal tract immunity and lentiviral acquisition across the epithelial barrier.

202 FULLY QUANTITATIVE PET IMAGING UNRAVELS THE RELATIVE SIZE OF GUT CD4 POOL

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Background: Previous studies have yielded conflicting results regarding the contribution of the gut to the overall CD4+ T-cell pool. SPECT imaging of whole-body CD4-pool in monkeys (by our group) using an anti-CD4 antibody (mAb) fragment (CD4R1-F(ab)'2) radiolabeled with Technetium-99m revealed low CD4-pool in the small and large intestines, corroborated by ex-vivo data. In contrast, PET imaging (by others) using the same anti-CD4R1 fragment radiolabeled with Copper-64 revealed substantial uptake in the gut of healthy animals and reduced uptake following SIV infection. The probe uptake in the clusters of lymph nodes and the spleen were similar between the two studies. Using a novel PET camera designed for monkeys (Mediso) with higher spatial resolution compared to clinical SPECT and PET cameras, we have repeated our experiments with the same anti-CD4R1 fragment radiolabeled with Zirconium-89 (89Zr).

Methods: The CD4R1-F(ab)'2 was radiolabeled with 89Zr using desferrioxamine and an isothiocyanate linker. Probe affinity was tested in MT4 cells. Healthy animals and reduced uptake following SIV infection. The probe uptake in the clusters of lymph nodes and the spleen were similar between the two studies. Using a novel PET camera designed for monkeys (Mediso) with higher spatial resolution compared to clinical SPECT and PET cameras, we have repeated our experiments with the same anti-CD4R1 fragment radiolabeled with Zirconium-89 (89Zr).

Results: We provide the first characterization of successfully reconstituted γδ T cell subsets in the peripheral blood and lymphoid tissue of BLT huMice, and we demonstrate an HIV-associated depletion of Vδ2 T cells and increase in Vδ1 T cells in the blood following infection. The functionality of human Vδ2 T cells isolated from the murine spleen of huMice was confirmed via ex vivo activation and proliferation with exposure to allogeneic monocytes, zoledronate, and IL-2. Unexpectedly, the adoptive transfer of expanded allogeneic Vδ2 T cells from uninfected human donors resulted in an increase in HIV viremia in HIV-infected BLT huMice. We determined that the in vivo exposure of the huMice to HIV resulted in the upregulation of CD4 expression on the human Vδ2 T cells and their direct susceptibility to HIV infection.

Conclusion: The robust reconstitution of γδ T cells can be achieved in BLT huMice for studying HIV and γδ T cell-based therapies targeting human diseases.
change in the setting of SIV infection. These data suggest that the prevalent notion of the gut as a major reservoir of the CD4 pool in the body needs to be revised.

203 PRE-ART VIRAL LOAD IS PREDICTIVE OF LYMPH NODE ART LEVELS IN SIV-INFECTED MACAQUES

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Background: Using dual-photon in-vivo imaging, we previously showed significantly lower concentrations of tenofovir (TFV) in rat lymph nodes (LN) compared to peripheral blood (PB). This finding was later confirmed for human and macaque models showing the IADM loading of ART corresponded with increasing IADM levels in LNMC and to a lesser extent PBMC. Given the human and macaque models showing the IADM loading rates during ART. A larger reduction in PB %Ki67+ in CD4T cells during M1 was observed compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.01). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 12-fold, M3: 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and LNMC compared to PBMC (median; M1: 3 and 2 and 7-fold, respectively; p≤0.005). Between M1 and M3, viral RNA decreased in LN, and IADM levels increased in both LNMC (2.3-fold, p≤0.001) and PBMC (1.4-1.5-fold, p≤0.03). LNMC IADM levels at M1 were inversely correlated with plasma viremia (TFVdp: p=0.003, FTCtp: p=0.07, LN (TFVdp: p=0.06, FTCtp: p<0.05) and rectal tissue (p<0.05) viral load at ART initiation, but not collagen deposition or PB %Ki67+. Yet, LNMC IADM levels at M1 were not predictive of viral decay rates during ART. A larger reduction in PB %Ki67+ in CD4T cells during M1 was associated with higher LNMC IADM levels at M1 (p<0.05).

Conclusion: NRTI IADM levels were consistently lower in LNMC vs. PBMC of SIV-infected and ART-treated RMs. Viral clearance during the first 3 months of ART corresponded with increasing IADM levels in LNMC and to a lesser extent in PBMC. Given the human and macaque models showing the IADM loading phase of ~1 week in PBMC and rectal tissue, these data suggest a link between viral dissemination/viral-induced generalized immune activation and drug penetration in LNs, a major reservoir of viral replication.

204 ALTERED RESPONSE PATTERN FOLLOWING NONCANONICAL NF-κB ACTIVATION IN INFANT MACAQUES

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Background: Strategies to eradicate or control the persistent HIV reservoir, the major obstacle to cure, would be highly beneficial for the 1.8 million children living with HIV. The “shock and kill” approach combines a latency reversing agent (LRA) to reactivate CD4+ T cells harboring integrated HIV-1 DNA with a clearance agent to enhance the immune-mediated elimination of infected, reactivated cells. Recent work in our lab identified a mimetic of the second mitochondrial-derived activator of caspases (SMACm), AZD5582, as an effective LRA in adult rhesus macaques. LRAs have not yet been evaluated in pediatric clinical or preclinical studies.

Methods: We evaluated the SMACm AZD5582 in 8 SIV-infected, ART-suppressed infant rhesus macaques (RM) compared to 4 ART-only controls. Infants were orally challenged with SIVmac251 at 4 weeks of age and treated with a suppressive triple ART regimen for over 1 year beginning 4 weeks post infection. SMACm treated animals received 10 weekly doses of AZD5582 i.v. at 100 ug/kg. Viral loads, Ki67 expression on CD4+ T cells, and AZD5582 concentrations were measured longitudinally to assess AZD5582 response.

Results: SMACm treated infants had similar viral loads at ART initiation as adult RMs that showed on-ART viremia following AZD5582 treatment (p = 0.72). Treatment with AZD5582 during ART was safe in our pediatric model, with no adverse clinical events. A significant increase in Ki67 expression on memory CD4+ T cells was observed 3d post-dose 1, 3, and 6 (p = 0.04). The first incidence of viremia >60 copies/ml was observed 3d following dose 4 with transient viremia observed in 5/8 treated RMs (63%, max = 771 copies/ml). Out of 135 viral load measurements performed on the 8 RMs that exhibited on-ART viremia during AZD5582 treatment, 8 were >60 copies/ml (6%), lower than the 46% we have observed in adult RMs. Plasma concentrations of AZD5582 indicate altered pharmacokinetics in infants compared to adults (Cmin = 294 ng/ml vs 756 ng/ml, respectively).

Conclusion: In summary, we have demonstrated that despite similar predicted reservoir size and expected pharmacodynamics, alterations in the pharmacokinetic profile of AZD5582 may lead to damped latency reversal in infant RMs. These results support a growing body of evidence that distinctions in the pediatric viral reservoir may result in divergent or blunted responses to LRAs in HIV-1-infected children and highlight the importance of pediatric models to evaluate HIV-1 cure interventions.

205 TIMELY ACQUISITION OF MYELOID-CELL IMMUNE-REGULATORY PHENOTYPE AND COVID-19 OUTCOME

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Background: Uncontrolled inflammatory responses, ranging from cytokine storm to immune-paralysis were described in COVID-19 worse prognosis.
Patients with an aggressive course are the bottleneck of COVID-19 pandemic management, and there is urgent need of understanding the underlying mechanisms to guide clinical decisions. Myeloid cell activation is likely a key player in SARS-CoV-2 infection.

**Methods:** Here, we longitudinally evaluated COVID-19 patients with respiratory insufficiency admitted to Hospital Santa Maria (Lisbon, Portugal), comparing those that did not require intensive care admission (NO-ICU) with those requiring high flux oxygen and/or mechanical ventilation (ICU). At each time point, an ex-vivo immune-phenotype by flow cytometry was analysed with both supervised and unsupervised approaches and clustering analysis of circulating cell subsets of monocytes (Mo) and dendritic cells (DCs), in parallel with specific antibody responses and a wide array of inflammatory mediators.

**Results:** Contrarily to other systemic viral infections, we found that COVID-19 patients with respiratory insufficiency featured systemic immune-suppressive/regulatory myeloid cell responses. Specifically, we observed a global reduction of CD14lowCD16+ Mo, and reduced expression of CD80, CD86, and SLAN. Contemporaneously, both Mo and DC showed increased expression of CD163, CD204, CD206 and PD-1+ immune-regulatory markers. Moreover, DCs, pDCs and basophils were significantly reduced. Inflammatory cytokines and chemokines associated with myeloid cell chemotaxis correlated with the phenotype changes. In NO-ICU patients (n=9) we observed a peak of these alterations at admission and a progressive regression to healthy phenotype at hospital discharge (as compared to age-matched controls, n=11). On the other hand, in ICU patients (n=11), the expression of immune-suppressive markers progressively increased until discharge. Notably, they featured significant reduction of HLA-DRhighPD-1+ and expansion of CD80-CD86- classical Mo and an increase of HLA-DRhighPD-1+ cells in Mo and in all DC subsets at recovery.

**Conclusion:** Altogether, these data favour an alternative view of a beneficial role of suppressive/regulatory myeloid responses in coping with COVID-19 pathogenesis. To further support our hypothesis, we are currently collecting data on lung injury and SARS-CoV-2 virome to correlate with the kinetics of myeloid responses.

### 206 SUPPRESSION OF ACE2 FUNCTION AND ANTIVIRAL IMMUNE RESPONSE BY SARS-CoV-2 INFECTION

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**Background:** SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) is also a protective factor that contributes to reduce inflammation and fibrosis in tissues. An active form of ACE2 can be released from the cell surface by host proteases ADAM17 and TMRPSS2, being the latter also necessary for viral entry. Due to its properties, the administration of soluble recombinant ACE2 has been proposed as a SARS-CoV-2 treatment. Here, we assess the role of ACE2 activity and antiviral immune response at the site of infection in nasopharyngeal swabs of SARS-CoV-2 patients, to unravel its effect on inflammation cascade and infection outcome.

**Methods:** Soluble enzymatic activity of ACE2 was measured in nasopharyngeal swabs at the time of PCR positivity (mean time from symptom=4d) and 3 days after in a cohort of mild SARS-CoV-2 patients (n=40, mean age=42y) and in uninfected controls. Gene expression profiles of ACE2, its proteases, ADAM17 and TMRPSS2, and interferon-stimulated genes (ISGs), DDX58, CXCL10 and IL-6 were also evaluated by RT-qPCR.

**Results:** Both ACE2 activity and mRNA expression decreased significantly during infection course in paired samples of SARS-CoV-2 infected subjects (p=0.048 and p<0.001, respectively), although differences between infected and uninfected subjects were only seen at mRNA level (p<0.001). Importantly, both ACE2 activity and mRNA expression showed a positive correlation with viral load (rho=0.352, p-value=0.0259), suggesting that viral infection is influencing ACE2 function. Similarly, infection downregulates TMRPSS2 expression (p-value<0.01), but not ADAM17, further indicating the viral-induced regulation of host receptors. In contrast to ACE2 data, a clear induction of IFN-stimulated genes, CXCL10, IL-6 and DDX58 (RIG-I), is observed upon infection (p-value<0.05 in all cases), demonstrating that SARS-CoV-2 induces an antiviral response and suggesting that ACE2 is not an ISG. This increased expression of ISGs is directly linked to viral load (rho=0.677, p-value<0.0001; rho=0.4026, p-value=0.0110; rho=0.3024, p-value=0.0613, respectively) but it is rapidly reversed during infection course.

**Conclusion:** Overall, our results demonstrate the existence of mechanisms by which SARS-CoV-2 suppresses ACE2 expression and function, intracellular viral detection and subsequent ISG induction, offering new insights into ACE2 dynamics and inflammatory response in the human upper respiratory tract that may contribute to understand the early antiviral host response to SARS-CoV-2 infection.

### 207 FUNCTION, HOMING, AND RESIDENCY OF T-CELL IMMUNE RESPONSES AGAINST SARS-CoV-2

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**Background:** In order to inform vaccine development on the correlates of protection against SARS-CoV-2, we performed detailed phenotypic and functional analyses in clinically-defined groups of patients recruited during the first wave of SARS-CoV-2 infection, including the assessment of Resident Memory T cells (TRM) in lung of convalescent patients.

**Methods:** Blood samples from 46 participants diagnosed with acute COVID-19 (14 symptomatic non-hospitalized; 20 mild-hospitalized and 12 severe-hospitalized) were obtained 7-16 days after symptoms onset. Lung biopsies were obtained from three convalescent patients 1 to 7.5 months after infection. The phenotype and functional capabilities of SARS-CoV-2-specific CD4+ and CD8+ T cells were measured by FACS after stimulation with a pool of overlapping SARS-CoV-2 viral peptides (M, N and S).

**Results:** Pattern variations associated with viral-specific T cell responses where based on two factors, the targeted viral protein and the cohort of patients assessed. Overall, stimulation with M and N viral peptides induced a Th1 profile exemplified by IFNγ production in CD4+ T cells and degranulation in CD8+ T cells respectively, whereas S peptides induced a Th2 profile exemplified by IL-4. Hospitalized patients showed increased IFNγ secretion in CD4+T cells in response to any viral protein compared to non-hospitalized patients (p=0.020 for M and S peptides in the mild group; p=0.004 for M, p=0.011 for N and p=0.007 for S peptides in the severe group; Figure 1) and IL-4 secretion in CD8+T cells in response to S peptides (p=0.004 and p=0.003 for mild and severe patients, respectively). In contrast, the expression of IL-10, which was mostly expressed in CR7+ cells, was significantly increased in CD4+T cells from non-hospitalized patients after stimulation with M peptides when compared to the mild COVID-19 group (p=0.035). Importantly, SARS-CoV-2 specific T cell responses with a biased TRM profile were detected up to 7.5 months after infection in the lung of convalescent patients. However, tissue responses strongly differed from blood.

**Conclusion:** Our results suggest that a balanced anti-inflammatory antiviral response promoted by non-spike proteins may be key to favor infection resolution without major complications. Further, while immune responses migrate and establish in the lung as resident memory T cells, the magnitude and profile of the lung SARS-CoV-2 specific T cells strongly differ from the response detected in blood.
208 COMPARTMENTAL T-CELL PROFILE AND IFN RESPONSE IN SARS-CoV-2–INFECTED SUBJECTS

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1Sapienza University of Rome, Rome, Italy

Background: A severe SARS-CoV-2 related immunopathology may be the driver cause underlying the deleterious clinical manifestations observed in COVID-19 patients. To identify possible tissue-specific immune responses patterns, a compartmental immunophenotyping analysis of CD4+ and CD8+ T lymphocytes and IFN response has been performed in SARS-CoV-2 infected subjects with acute respiratory distress syndrome.

Methods: Bronchoalveolar lavage (BAL) and Peripheral Blood Mononuclear Cells (PBMC) samples were collected from 13 SARS-CoV-2 infected subjects (9 males and 4 females) consecutively admitted to intensive care unit (ICU) of Policlinico Umberto I, Sapienza University Hospital in Rome (Italy). The frequencies of CD4+, CD8+ T lymphocytes and those expressing immune activation markers (CD38, HLADR), naive, central memory (CMEM), and effector memory (TEM) T cell subsets were evaluated in both anatomical sites by multiparametric flow cytometry. Gene expression levels of interferon regulatory factor 7 (IRF7) and the Interferon Stimulated Gene 15 (ISG15) were evaluated in BAL and PBMC by Real-time PCR.

Results: Critically SARS-CoV-2 infected patients exhibited a lung compartmentalization of CD8+ T cells (p<0.003), with a lower CD4/CD8 ratio in BAL compared to blood district (p<0.01). However, higher frequencies of CD8+ T cells were recorded in PBMC of female SARS-CoV-2 infected patients (p<0.04) and the same trend was observed in the lung compartment. By contrast, a trend of increasing CD4+ T cells frequencies was observed in BAL samples of male patients, as opposed to blood compartment. Additionally, an increased expression of immune activation markers CD38 and HLADR has been detected in BAL CD8+ T cells (p<0.01) as well as in blood CD4+ T cells (p=0.03). An increased frequency of CD4+ and CD8+ TEM has been documented in BAL of SARS-CoV-2 infected patients (p<0.05), as opposed to higher frequencies of CD4+ and CD8+ TEM cells recorded in the blood compartment (p<0.01). Notably, higher levels of ISG15 and IRF7 found in BAL were inversely associated to activated CD8+ T cell frequencies in the lung compartment compared to blood district (ISG15: r=0.570, p=0.05) (IRF7: r=-0.683, p=0.01).

Conclusion: Our findings provide new insight into a distinct T cell profile and IFN genes expression in the lung and in the blood compartment of SARS-CoV-2 infected patients, that might be highly relevant for the clinical course of COVID-19.

209 HOST FACTORS ASSOCIATED WITH PERSISTENT SARS-CoV-2 VIRAL RNA IN COVID-19 OUTPATIENTS

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Background: Sustained molecular detection of SARS-CoV-2 RNA in the upper respiratory tract (URT) is common and confounds infection control efforts in the community. The mean duration of viral RNA detection is ~17 days, and ~14% of people with mild or no symptoms have detectable viral RNA for >4 weeks. Understanding the kinetics of early immune responses to SARS-CoV-2 infection is critical to identify potential biomarkers of disease outcome. A myriad of soluble mediators including, pro-inflammatory, immune-suppressors and growth factors, play a relevant role in the disease progression. However, to date, limited data is available about the role of soluble factors and most studies focus only in severe cases with limited follow-up. Here, we studied with high resolution the kinetics of soluble mediators in mild to moderate cases of SARS-CoV-2 infection 1-90 days from symptom onset (DFS0).

Methods: We identified a core signature including 19 highly correlated soluble factors at 1-14 DFS0, based on clustering analysis. The core signature contained CD40L and GROb, #2 (G-CSF, PDL1-B7, Fractalkine, IL8, IL7, IL6, and VEGF association). Moreover, by 60-75 DFS0, the cluster composition between 1-14 and 30-45 DFS0, due to the loss of PDL1-B7, IL10) and #3 (IL7, IL6, and VEGF). We found major changes in #2 and #3 within 1-14 DFS0. IgG and IgM levels were determined by ELISA. We selected a multiplex assay including 45 soluble human factors.

Results: See Table for participant characteristics. Of 56 participants with observed viral RNA clearance, mean time to clearance was 33.5 days. The hazard ratio for obesity vs overweight/normal weight was 0.37 (95% CI 0.18-0.78, p=0.009). Elevated mucosal SARS-CoV-2-specific IgG did not associate with faster viral RNA clearance. The maximum time from symptom onset to virus culture positive sample was 12 days, which is just after the mean time of first positive mucosal SARS-CoV-2-specific IgG detection.

Conclusion: Obesity is associated with prolonged SARS-CoV-2 RNA detection in outpatients. Mucosal SARS-CoV-2 IgG is not associated with faster clearance of viral RNA from the URT, suggesting that viral clearance is mediated by select host immune responses.
addition, we observed a negative correlation between IgG and IgM levels with IL4 production at 1-14 DfSO (IgG: \( p = -0.82, p=0.012 \); IgM: \( p=-0.83, p=0.011 \)). Similarly, a negative correlation was observed between IgG and Mip3a at 30-45 DfSO (IgG: \( p =-0.78, p=0.023 \); IgM: \( p =-0.81, p=0.022 \)).

**Conclusion:** We delineated a core signature of soluble factors in mild to moderate SARS-CoV-2 infection, including growth factors, chemokines and pro-inflammatory cytokines. The longitudinal follow-up of this signature revealed significant changes during the 1-90 DfSO. This information can provide new insights for the definition of biomarkers for patient stratification in mild or moderate SARS-CoV-2 infection. Further data is needed to understand the association between IL4 and Mip3a with low IgG levels.

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**First high-dimensional examination of intestinal biopsies in patients with COVID-19**

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**Background:** SARS-CoV-2, the etiopathological agent for COVID-19, engages host ACE2 receptor for cellular entry. The brush border of the small intestines express high levels of ACE2 in physiological conditions. Gastrointestinal (GI) manifestations are common among COVID-19 patients. However, to date, there is limited information regarding intestinal response to SARS-CoV-2 infection.

**Methods:** Intestinal biopsies were obtained from 17 COVID-19 patients (17.3-17.5 days from the last positive nasal swab) for cellular and transcriptomic analyses using mass cytometry and RNA-sequencing. Ten COVID-uninfected individuals served as controls. The epithelial compartment (EC) and lamina propria (LP) were analyzed separately.

**Results:** The cellular profiles of intestinal tissues from COVID-19 patients showed reduced frequencies of CD206+ CDC2s and plasmacytoid dendritic cells in the LP of COVID-19 patients by mass cytometry. Effector T cell (PD1+CD38+) frequency was increased in the LP and blood of COVID-19 patients. Intraepithelial lymphocytes (IEL) were increased in the EC of COVID-19 patients, with a concomitant decrease in CD206+ CDC2s. RNA sequencing revealed an active downregulation of genes involved in inflammatory pathways including Th17 and IBD-associated pathways, while an upregulation of intestinal barrier function (mucin biosynthesis), amino acid metabolism and mineral absorption pathways was noted. Gene expression of Neuropilin-1 (NRP-1), a putative SARS-CoV-2 receptor as well as key inflammatory cytokines (IL-18, IFNγ, CCL24 and CXCL8) were significantly reduced in COVID-19 patients compared to controls. A low intensity antiviral host response signature was observed predominantly in EC reflecting the cellular localization of the virus.

**Conclusion:** Epithelial, myeloid and lymphoid cell alterations characterize intestinal response to SARS-CoV-2 infection with an unanticipated downregulation of key inflammatory pathways that have been implicated in adverse outcomes associated with COVID-19. These data stand in contrast to reports from the pulmonary and systemic compartments and identify a potential mitigating role of the GI tract in COVID-19-associated immunopathology.
SARS-CoV-2 ex vivo and in vivo and suggest that β-cell infection may contribute to pancreatic dysregulation observed in COVID-19 patients.

Results: Participants with Moderate and Severe COVID-19 presentations were older compared to the Mild group (p<0.001) (Mild: 42.2 years (range: 20 – 63 years), Moderate: 64.2 years (range: 33 – 97 years); Severe: 61.9 years (range: 32 – 86 years)). The Severe group had a greater proportion of men (69% vs 36%) than women and a greater proportion of black/African Americans (27% vs 6%) than whites versus the Mild group. IfABP, LBP, and sCD14 levels were significantly higher in participants with Moderate or Severe disease compared to Mild disease (Table 1), with no significant differences between Moderate and Severe groups. Among the 65 participants with samples from two timepoints (mean separation of 24.3 +/- 22.4 days), sCD14, IfABP, and LBP did not change significantly.

Conclusion: Levels of biomarkers of enterocyte turnover (IfABP), microbial translocation (LBP), and lipopolysaccharide-induced monocyte activation (sCD14) were increased in patients with Moderate and Severe COVID-19 compared to Mild COVID-19. Whether interventions that improve gut health will attenuate the cytokine storm that precipitats Severe COVID-19 needs further study.

<table>
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<td>177.5 (66.5)</td>
<td>251.7 (43.4)</td>
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213 SARS-CoV-2 INFECTIONS AND REPLICATES IN CELLS OF THE ENDOCRINE AND EXOCRINE PANCREAS
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Background: The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), mainly affects the lung, but may also result in extrapulmonary manifestations such as lesions in kidneys, heart, brain, gastrointestinal and endocrine organs. Clinical data suggest that a SARS-CoV-2 infection disturbs glucose homeostasis, and cases of new-onset type 1 diabetes mellitus after SARS-CoV-2 infection have been reported. However, experimental evidence that SARS-CoV-2 can infect pancreatic tissue is lacking. We here explored whether pancreatic tissue is susceptible to SARS-CoV-2 infection.

Methods: We analyzed healthy human pancreas tissue and cells for ACE2 and TMPRSS2 expression by immunohistochemistry. We exposed human Langerhans islets to SARS-CoV-2 ex vivo and determined viral infection by staining for SARS-CoV-2 spike and nucleoprotein. Viral replication was monitored by detection of released viral RNA by qPCR and infectious titers by F DAT.

Results: We here explored whether pancreatic tissue is susceptible to SARS-CoV-2 infection in pancreatic cells, including those that stain positive for the β-cell marker NKX6.1.

Conclusion: Our data demonstrate that the human pancreas is a target of SARS-CoV-2 ex vivo and in vivo and suggest that β-cell infection may contribute to pancreatic dysregulation observed in COVID-19 patients.

214 UNIQUE CARDIOMETABOLIC IMMUNE SIGNATURES IN SEVERE COVID-19 PATIENTS AND SURVIVORS
Namal Liyanage1, Manjula Gunasena1, Yasaswi Wijewanthana1, Emily Bowman1, Janelle Gabriel1, Amrendra Kumar1, Aaren Kettelhut1, Anushka Ruwanpathirana1, Krishanthi Weragalaarachchi1, Dhania Kasturiratna1, Anna Vilgelms1, Joseph Bednash1, Thorsten Denzberg1, Nicholas Funderburg1
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Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a highly pathogenic coronavirus virus which causes COVID-19 and resulted in millions of deaths and led to a global public health emergency. SARS-CoV-2 infected patients exhibit a wide variety of clinical manifestations ranging from asymptomatic to severe complications and death. SARS-CoV-2 infection can lead to excessive immune activation, inflammation and multi-organ damage. Clinical data showed that COVID-19 may promote the development of cardiovascular disorders (CVDs). Immune activation, thrombosis, cytokine storm, and altered adhesion molecule expression on leukocyte populations, have been proposed as possible mechanisms that trigger COVID-19 associated CVDs. A lack of systematic studies on how SARS-CoV-2 infection triggered immune responses that may lead to CVDs, hinder early risk identification and therapeutic interventions.

Methods: In this study, by using deep immune cell profiling (high dimensional flowcytometry) in fresh whole blood and extensive plasma cytokine and chemokine profiling, we explore potential mechanisms that could lead to CVDs in severe COVID-19 patients that did not have previous known CVDs (ICU) (n=20) as well as patients recovered from COVID-19 (RD) (n=30) compared to healthy donors (n=17). To identify the major statistically significant immune signatures that predict CVD risk in ICU patients and RD, we performed parametric (ANOVA) and non-parametric (Kruskal–Wallis) statistical tests with Dunn’s and Tukey’s post hoc tests. Integrative correlation and network analysis were performed by computing Spearman’s coefficients. Correlations with r > 0.3, r < −0.3 and P < 0.01 were considered significant.

Results: We found that significantly elevated eosiophilin, neutrophils and increased circulating levels of tissue factor, fatty acid binding protein 4 and, LPS binding protein in ICU patients suggested increased immune activation and thrombotic risk. Interestingly, we found significant elevation of several immune parameters (TIMP-1, TIMP-2, M-CSF, Monocytes) that were associated with cardiometabolic risk, even 3-4 months after the recovery of initial COVID-19 infection in RD. Furthermore, we found unique relationship with cytokine and cellular responses in ICU and RD groups compare with HD.

Conclusion: Our data strongly suggest a possible mechanistic link between SARS-CoV-2 induced dysregulated immune responses and increased cardiometabolic risk in severe COVID-19 patients.

215 IL10 AND B CELLS COOPERATE TO PREDICT SARS-COV-2 DISEASE SEVERITY
Susan Pereira Ribeiro1, Jozefien De Clercq2, Sarah Gerlo1, Ashish A. Sharma1, Lynong Mao1, Ashish A. Sharma1, Adam N. Pelletier1, Basel Col1, Marion Pardons2, Sarah Gerlo1, Anna Bruche1, Robert Balderas2, Martin Guilliams2, Linos Vandekeerkhove2
1Emory University, Atlanta, GA, USA, 2Ghent University, Ghent, Belgium, 3Case Western Reserve University, Cleveland, OH, USA, 4BIO Biosciences, San Francisco, CA, USA

Background: SARS-COV-2 has infected more than 62 million people worldwide and led to almost 1.5 million deaths. Exacerbated inflammation, lymphopenia and coagulopathy are part of the complex cascade of events that can lead to death. Additionally, elevated B cells, antibody production and heightened IL-10 levels were associated with severe disease.

Methods: We performed scRNAseq/ITEseq on matched peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage (BAL) from 5 COVID-19 positive donors with different disease outcomes at time of hospitalization. B cell gene signatures and cytokine/chemokine levels in plasma and BAL fluid (BALF) were associated to live neutralizing antibodies (NAbs) as well as to viral load (VL) readouts.

Results: Fourteen clusters of B cells were identified in PBMCs. They included subsets of naïve, memory B cells and plasmablasts. Ten clusters, comprising mature effector B cells, were found in BAL while no naïve B cells were observed. Titers of NAbs in the BALF were significantly associated to memory B cell signatures associated to chemokine signaling which was positively correlated to viral load (VL) readouts.

Conclusion: Studies on how SARS-CoV-2 infection triggered immune responses that may lead to CVDs, hinder early risk identification and therapeutic interventions.
the chemokines measured in BALF. This suggests that mature B cells in PBMCs could migrate from periphery to the lung to counteract infection. Importantly, B cells in COVID19 patients presented a strong inflammatory signature that included heightened levels of interferons and IL-10 signaling, the latter a marker of disease severity known to promote B cell differentiation and antibody production.

Conclusion: While this work corroborates prior findings in the literature, it associates IL10 levels and B cell migration to disease severity. A detailed analysis of the antibody repertoire and B cell clonality induced upon infection is under way and would support the understanding of the role of B cells in SARS-CoV-2 pathogenesis and also lead to possible immune interventions to counteract severity.

216 RECRUITMENT OF SPECIFIC MONOCYTE AND DC SUBSETS TO THE LUNG DURING SEVERE COVID-19
Ildefonso S. Cerrillo1, Pedro Landete2, Ignacio De Los Santos2, Hortensia De La Fuente1, Maria J. Calzada1, Isidoro Gonzalez1, Arantzuza Alfranca1, Francisco Sanchez1, Cecilia Munoz2, Joan Soriano1, Julio An ociea1, Enrique Martin-Gay1, for the REINMUN COVID Research Group
1Hospital Universitario de La Princesa, Madrid, Spain, 2Universidad Autónoma de Madrid, Madrid, Spain

Background: SARS-CoV-2 is responsible for the development of COVID-19 in infected individuals, who can exhibit mild to severe symptoms including acute respiratory distress syndrome (ARDS). Critical patients developing ARDS are characterized by exacerbated inflammation and dysregulated adaptive immune responses. However, the differential association of specific myeloid subsets of dendritic cells (DC) and monocytes (Mo) to the induction of ARDS in these critical COVID-19 patients is poorly understood.

Methods: A total of 64 COVID-19 patients qPCR positive for SARS-CoV were included in the study. Patients were stratified into 3 subgroups attending to non-severe (G1), severe (G2) and critical (G3) severity based on changes in respiratory frequency (RF), partial pressure arterial oxygen and fraction of inspired oxygen ratio (PaO2/FiO2) and respiratory failure values. PBMC were obtained from blood samples collected from all COVID-19 patient subgroups and with paired cell infiltrates from bronchial aspirates in the case of critical G3 patients requiring respiratory support. PBMC samples from n=22 non-COVID individuals were also included for comparison purposes. Expression of markers defining different DC and Mo subsets and their level of activation were analyzed by multicolor flow cytometry.

Results: CD141+ conventional and CD123hi plasmacytoid DCs were similarly depleted from blood in COVID19 patients (p<0.0001; p<0.0001) from all severity subgroups but were absent in the lung infiltrates from critical individuals. In contrast, CD1c+ DC, inflammatory transitional and non-classical Mo were dramatically depleted from the blood and preferentially enriched in lung infiltrates in patients with critical G3 COVID-19 individuals (p<0.01; p<0.01). Remarkably, proportions of transitional Mo in the blood were increased in G1 COVID19 patients exhibiting non-severe progression. Importantly, myeloid subsets infiltrating the lung of critical G3 COVID19 patients were characterized by increased expression of the activation marker CD40. Moreover, activated CD103+ CXCR5+ and CD103+ CXCR5+ CD8+ T cells were enriched in the lungs from critical COVID-19 patients (p<0.01; p<0.05).

Finally, higher levels of CD40 on transitional Mo were positively associated with proportions of CD103+ CXCR5+ CD8+ T cells in the lung.

Conclusion: The study identified the recruitment of specific myeloid subsets from the blood to the lung in critical COVID-19 patient that could be targeted by future therapies.

217 SARS-CoV-2 ORF3a ACTIVATES THE NLRP3 INFLAMMASOME
Kimberly E. Rousseau1, Alexis Figueuroa1, Guido Massaccesi1, Michael Chatterton1
1Johns Hopkins University, Baltimore, MD, USA

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of Coronavirus Disease 2019 (COVID-19), began circulating in humans in late 2019 and has since spread to pandemic levels. Numerous clinical studies into the inflammatory syndrome in critically ill COVID-19 patients have highlighted aberrant immune responses and levels of pro-inflammatory cytokines. Among the dysregulated pro-inflammatory cytokines are interleukin (IL)-1β and IL-18. These two cytokines are produced after the activation and assembly of inflammasomes, multi-protein structures of the innate immune system that recognize and respond to traumas such as viral infection, tissue damage, and other stimuli. The purpose of this study is to define the viral trigger for inflammasome activation by SARS-CoV-2 and to understand its mechanism for activation.

Methods: In this study, we employed a transfection reconstitution system to study the structural and non-structural components of SARS-CoV-2 for inflammasome activation. Plasmids encoding the inflammasome components and individual plasmids encoding each SARS-CoV-2 protein of interest were co-transfected, allowing us to probe the relationship between these viral proteins and the NLRP3 inflammasome.

Results: We report that the accessory protein open reading frame (ORF) 3a is sufficient to trigger assembly of the NLRP3 inflammasome and production of mature IL-1β. Among the 26 viral proteins assayed, non-structural protein (nsp) 2 and ORF3b also triggered NLRP3 inflammasome activity and release of mature IL-1β, though to a less extent. MCC950, a selective small molecule inhibitor of the ATPase function of NLRP3, reduces IL-1β production in response to these viral proteins to mock levels.

Conclusion: The identification of SARS-CoV-2 ORF3a as the viral protein responsible for NLRP3 inflammasome activation is an important step in understanding the inflammatory response seen in severe COVID-19 disease. Further study into the mechanism of this event has the potential to highlight clinical targets for drug interventions in severe cases of the disease.

218 COLONIC GRANZYME B+ CD4 T CELLS ASSOCIATE WITH GUT AND SYSTEMIC T-CELL ACTIVATION
Stephanie Dillon1, Emily Cooper1, Tezha Thompson1, Kaylee Mickens1, Kejun Guo1, Cheyret Wood1, Katerina Kechris1, Mario Santiago1, Carla Wilson1
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Background: Alterations in gut homeostasis and changes in the microbiome (dysbiosis) have been linked to features of pathogenesis and comorbidity events in people with HIV (PWH). We reported human gut lamina propria (LP) CD4 T cells express the cytolytic molecule Granzyme B (GZB) following in vitro exposure to commensal enteric bacteria with enhanced expression in the presence of HIV-1. Here, we investigated CD4 T cell GZB expression in PWH and uninfected controls and explored its relationship with in vivo markers of HIV-1 pathogenesis and gut microbiome.

Methods: GZB+ CD8+ (due to downregulation of CD4 by HIV-1) T cells were enumerated in archived colon tissue (PMID24399150) from 10 untreated, chronically-infected PWH and 10 uninfected controls using multispectral imaging. GZB+ CD4 T cells express the cytolytic molecule Granzyme B (GZB) following in vitro exposure to commensal enteric bacteria with enhanced expression in the presence of HIV-1. Here, we investigated CD4 T cell GZB expression in PWH and uninfected controls and explored its relationship with in vivo markers of HIV-1 pathogenesis and gut microbiome.

Results: GZB+ CD8+ T cells, as well as GZB+ CD4 T cells, were depleted from PWH compared to uninfected controls (p<0.05). In PWH, GZB+ CD8+ T cells were also significantly associated with markers of systemic inflammation, including C-reactive protein and IL-6 levels (p<0.05). In contrast, GZB+ CD4 T cells in uninfected controls were positively associated with markers of gut inflammation, such as IL-17 and IL-15 levels (p<0.05). These findings suggest that GZB+ CD4 T cells are enriched in the gut of PWH and may contribute to the dysregulated immune response seen in this population.

Conclusion: The study identified the recruitment of specific myeloid subsets from the blood to the lung in critical COVID-19 patient that could be targeted by future therapies.

217 SARS-CoV-2 ORF3a ACTIVATES THE NLRP3 INFLAMMASOME
Kimberly E. Rousseau1, Alexis Figueuroa1, Guido Massaccesi1, Michael Chatterton1
1Johns Hopkins University, Baltimore, MD, USA

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of Coronavirus Disease 2019 (COVID-19), began circulating in humans in late 2019 and has since spread to pandemic levels. Numerous clinical studies into the inflammatory syndrome in critically ill COVID-19 patients have highlighted aberrant immune responses and levels of pro-inflammatory cytokines. Among the dysregulated pro-inflammatory cytokines are interleukin (IL)-1β and IL-18. These two cytokines are produced after the activation and assembly of inflammasomes, multi-protein structures of the innate immune system that recognize and respond to traumas such as viral infection, tissue damage, and other stimuli. The purpose of this study is to define the viral trigger for inflammasome activation by SARS-CoV-2 and to understand its mechanism for activation.

Methods: In this study, we employed a transfection reconstitution system to study the structural and non-structural components of SARS-CoV-2 for inflammasome activation. Plasmids encoding the inflammasome components and individual plasmids encoding each SARS-CoV-2 protein of interest were co-transfected, allowing us to probe the relationship between these viral proteins and the NLRP3 inflammasome.

Results: We report that the accessory protein open reading frame (ORF) 3a is sufficient to trigger assembly of the NLRP3 inflammasome and production of mature IL-1β. Among the 26 viral proteins assayed, non-structural protein (nsp) 2 and ORF3b also triggered NLRP3 inflammasome activity and release of mature IL-1β, though to a less extent. MCC950, a selective small molecule inhibitor of the ATPase function of NLRP3, reduces IL-1β production in response to these viral proteins to mock levels.

Conclusion: The identification of SARS-CoV-2 ORF3a as the viral protein responsible for NLRP3 inflammasome activation is an important step in understanding the inflammatory response seen in severe COVID-19 disease. Further study into the mechanism of this event has the potential to highlight clinical targets for drug interventions in severe cases of the disease.
219 ALTERED GASTROINTESTINAL PLASMA CELLS CONTRIBUTE TO DYSBIOSIS AND VIRAL REPLICATION

Francesca Cossarini1, Louise Leyre1, Divya Jha2, Minami Tokuyama1, Alexandra E. Livanos1, Michael Tankelevich1, Ivo SahBandar1, Ilaria Morgo1, Icahn School of Medicine at Mt Sinai, New York, NY, USA

Background: Intestinal dysbiosis is a feature of HIV-1 infection. Secreoty IgA mediates critical bidirectional interactions between the mucosal immune system and the intestinal microbiome. Here, we sought to better define the role played by mucosal IgA in mediating dysbiosis during chronic HIV-1 infection.

Methods: 11 HIV-infected viremic patients, 35 HIV-infected patients with 50 HIV-uninfected healthy controls were recruited to undergo ileocolonoscopy. Mucosal mononuclear cells were obtained using collagenase digestion of fresh biopsies. B cell subsets including plasma cells (PC) (defined as CD38highCD27+CD45+ live cells) were examined by multiparameter flow cytometry. Paraffin-preserved biopsy samples were examined via fluorescent microscopy co-staining IgA, CD18 and CDAP. Metagenomic sequencing of stool DNA was performed using Illumina HiSeq. The relative abundance of each microbe was obtained by metaphlan with differences quantified from cryo-preserved mucosal biopsies using qRT-PCR. Results: The number of LP GB+ CD4 T cells per tissue area were higher in PWH (median 39 GB+ CD8- T cells/mm², range 16-104) versus controls (17, 6-33; P=0.005) despite relative depletion of CD4 T cells (PWH: 400 CD8- T cells/mm², 226-622; controls: 930, 517-1090; P=0.001). Numbers of LP GB+ CD4 T cells were significantly associated with increasing PB and colon CD4 and CD8 T cell activation levels and RA of Prevotella species (Table 1). Percentages of PB CD4 T cells expressing GB were higher in PWH (8.7%, 0.8-15.1) versus controls (0.4%, 0.01-3.8; P=0.0006). By contrast, PB GB+ CD4 T cells did not associate with systemic or colon measurements or with microbiota RA.

Conclusion: GB+ CD4 T cells are enriched in colon tissue of untreated PWH. Colon, but not PB, GB+ CD4 T cells, associated with colon and systemic T cell activation, a predictor of disease progression, and with increasing RA of Prevotella species, commensal enteric bacteria previously linked to mucosal inflammation and T cell activation. These novel findings implicate GB+ CD4 T cells as potential key players in HIV-associated gut immune activation, processes potentially driven by dysbiotic bacteria.

Table 1. Linear models with number of colonic GB+CD4 T cells as the outcome

<table>
<thead>
<tr>
<th>Predictor (representing a different model fit)</th>
<th>Regression Slope Estimate (SE)</th>
<th>FDR*</th>
</tr>
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<tbody>
<tr>
<td>Blood T Cell activation</td>
<td></td>
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<tr>
<td>CD84HLA-DR+CD4 T cells (% of CD4 T cells)</td>
<td>16.3 (4.4)</td>
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</tr>
<tr>
<td>CD84+HLA-DR+CD4 T cells (mg tissue)</td>
<td>0.0002 (0.0004)</td>
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</tr>
<tr>
<td>CD84+HLA-DR+CD4 T cells (mg tissue)</td>
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<td>0.0022</td>
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<td>Mucosa-associated bacteria</td>
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<td></td>
</tr>
<tr>
<td>RA of Prevotella (% of total bacteria)</td>
<td>76.4 (16.8)</td>
<td>0.01</td>
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<tr>
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<td>0.03</td>
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<tr>
<td>RA of Prevotella copit (% of classified species)</td>
<td>79.6 (22.1)</td>
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*False Discovery Rate correcting for multiple comparisons. Microbiome variables (N=32) were assessed and corrected for separately from other markers of HIV-1 pathogenesis (N=21).

SE: Standard Error. RA: relative abundance.

220 HIV REBOUND IN CONTROLLERS IS ASSOCIATED WITH SPECIFIC FECAL MICROBE PROFILE

Yanhui Cai1, Steven G. Deeks2, Cynthia Brinson1, Moti Ramgopal1, Norman Jones3, Edwin DeJesus1, Anthony Mills1, Peter Shalit4, Brian Moldt1, Lishomwa5

Background: Intestinal dysbiosis and viral suppression correlate with increased immune activation levels and RA of Prevotella species (Table 1). Percentages of PB CD4 T cells expressing GB were higher in PWH (8.7%, 0.8-15.1) versus controls (0.4%, 0.01-3.8; P=0.0006). By contrast, PB GB+ CD4 T cells did not associate with systemic or colon measurements or with microbiota RA.

Conclusion: Depletion of IgA+ PC of viremic HIV patients might contribute to intestinal dysbiosis and is associated with higher tissue levels of HIV RNA.

Download Table 1. Linear models with number of colonic GB+CD4 T cells as the outcome

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SE: Standard Error. RA: relative abundance.
abundance at the phylum level were measured by Spearman’s rank correlation. A univariate Cox Proportional Hazard regression model explored the association between time to viral rebound and the abundance of microbiota species. Results: Prevotella copri was enriched in VC at baseline (p=0.0018), as well as in CHI (p=0.0024), compared to healthy volunteers. VES partially reversed this dysbiosis by decreasing Prevotella copri levels closer to those in healthy volunteers. Proteobacteria abundance was positively correlated with VES-induced elevation of 16Ss and K6G7+C44+ T cells (r=0.75, p=0.07). Higher abundance of fecal Ruminococcus gnavus at the pre-ART timepoint was associated with shorter time to viral rebound after ART in the overall study population (FDR adjusted p-value=0.014).

Conclusion: The enrichment of certain microbiome species may contribute to HIV persistence, as evidenced by faster rebound off ART. VES enhances the immune response and can potentially favorably alter the composition of the fecal microbiome. Further studies are needed to understand the mechanistic interactions between immune modulation and microbiome, and subsequent impacts on antiviral responses and HIV reservoir in cure studies.

222 A RANDOMIZED CONTROLLED TRIAL OF RIFAXIMIN IN INDIVIDUALS WITH HIV ON LONG-TERM ART
Kristi Huik1, James O. Virga1, Catherine Rehm2, Jennifer Bell1, Brian Luke1, Netanya S. Ulay1, Deborah McMahon1, Anuradha Ganesan1, Frank Maldarelli1
1National Cancer Institute, Frederick, MD, USA, 2National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 3Leidos Biomedical Research, Inc, Frederick, MD, USA, 4University of Texas Health Science Center at Houston, Houston, TX, USA, 5University of Pittsburgh, Pittsburgh, PA, USA

Background: Ongoing immune activation plays a key role in clinical outcomes of people living with HIV during long-term combination antiretroviral therapy (cART); the forces responsible for elevated immune activation (IA) remain uncertain. Microbial translocation from the gastrointestinal tract have been reported to contribute to immune activation, and a modest reduction of IA with use of the nonabsorbable antibiotic rifaximin in immune nonresponders, presumably by decreasing microbial translocation across the gut. However, the effects of rifaximin on the gut microbiome in HIV infected individuals remain uncertain. We recently conducted a randomized placebo-controlled trial of the effects of rifaximin on IA in individuals with HIV undergoing cART. Here we report analysis of the gut microbiome during rifaximin therapy and analyze the bacterial composition of stool obtained during the course of the study.

Methods: Individuals (N=42) receiving cART >3 years with HIV viremia <50 c/ml were enrolled in a placebo-controlled double blind crossover study of rifaximin. A panel of soluble and cellular markers of IA were measured throughout the study; the primary endpoint was the change in sCD14 levels during the placebo and rifaximin phases of the study. In addition, stool samples were obtained throughout the study and microbial communities were assessed based on 16S rRNA gene amplicon sequencing of day 0 and day 28 stool samples of each arm. Standard measures of microbial diversity, operational taxonomic units (OTUs) and Shannon entropy were determined, and the log ratios of these determinations on day 0 and day 28 were compared using Wilcoxon rank sum tests.

Results: 17 participants provided samples at the beginning and end of each phase; participants (10% female, 22% non-white) had median age 49 years, with a median duration of HIV infection of 20 years and entry CD4=302 cells/μL. Microbial communities were recovered from stool samples and 25,000 reads were available for analysis per sample. As expected, Firmicutes and Bacteroidetes phyla accounted for up to 90% of the microbial composition in each participant. A modest but significant decrease in OTUs (p=0.04) was detected in the rifaximin arm of the study; no significant changes in Shannon entropy were detected.

Conclusion: Short-term rifaximin therapy resulted in modest but significant change in the gut microbiome, but this shift did not result in downstream changes in IA.
a viral outgrowth assay. The isolate from TPSM replicated slightly better than an isolate from TPSF with p24 concentrations of 1441 ng/ml versus 655 ng/ml at day 10 compared to 44 ng/ml versus 23 ng/ml at day 0. The viral isolate from TPSM replicated better than the isolate from TPSF in humanized mice with viral loads of 1.46E and 3.76E copies/ml versus 9.66E and 2.56E copies/ml respectively at 7 days post-infection. Sequencing of LTR, gag, pol, integrase and the accessory genes confirmed that TPSF and TPSFM were a transmission pair. All four TPSF replication-competent viral isolates had numerous mutations and deletions in nef, including a 16 base pair deletion that resulted in a frame shift and premature stop codon. These mutations affected nef function as primary CD4+ T cells infected with TPSF virus had 2-4-fold downregulation of CD4 and HLA-A2 (mean fluorescence intensity [MFI] of 9644 and 3083) compared to TPSM virus infected cells (MFI of 3634 and 782 respectively).

Conclusion: Together, these data suggest that unlike TPSM, TPSF viral isolates are attenuated due to non-functional nef. This attenuation probably contributed to the natural control of viral replication in TPSF. Our data suggests that transmission of attenuated minor variants can explain discordant outcomes in some transmission pairs.

224 A META GENOME-WIDE ASSOCIATION STUDY OF HIV-DISEASE PROGRESSION IN HIV CONTROLLERS

Luis M Real1, María Sáez2, Anais Gorma-Gomez3, Antonio Gonzalez-Perez3, Christian A. Thorball, Rocío Ruiz, Reyes Jiimenez-Leon1, Alejandro Gonzalez-Serra1, María C. Gasca-Capote1, Alberto Perez-Gomez2, Jacques Fellay4, Mathias Lichterfeld5, Ezquiel Ruiz-Mateos6, for the Swiss HIV Cohort Study

Background: HIV-controllers are a heterogeneous group of individuals who maintain low or undetectable levels of viremia in the absence of antiretroviral treatment. Some of them eventually experience immunologic progression with marked CD4+ T-cell decline. Our aim was to identify genetic factors associated with avoiding CD4+ T-cell loss in HIV-controllers.

Methods: Retrospective case-control genome-wide association study (GWAS). We analyzed genotype data-sets within long-term non-progressor HIV-1 controllers (LTNP-Cs); who maintained CD4+ T-cells counts >500 cells/mm3, for more than 7 years after HIV-1 diagnosis vs non-LTNP-Cs who developed CD4+ T-cell counts <500 cells/mm3, belonging to both, the International HIV-controllers Study Cohort (ICSC) and Swiss HIV Cohort Study (SHCS). Plink was used to carry out individual GWAS and meta-GWAS, logistic regression procedures and fixed or random effect models were applied, respectively. Magma and Webgestalt softwares were used to carry out gene-based association studies with detection of multi-marker effects and enrichment analyses with multiple testing corrections, respectively.

Results: A total of 471 and 442 HIV-controllers were included from the SHCS and ICSC, respectively. Among them, 115 (24.2%) and 233 (52.7%) were LTNP-Cs, respectively. No SNP or gene association with the LTNP-C phenotype in the individual GWAS or in the meta-analysis after multiple testing corrections was found (Table 1). However, those SNPs previously associated with natural HIV control linked to HLA-B (rs2395029 [p=0.0004; OR=1.82], rs9266409 [p=0.003; OR=1.42], rs59440261 [p=0.00004; OR=1.97]), MICA (rs11224303 [p=0.0005; OR=1.35]) and PSORS1C1 loci (rs3815087 [p=0.026; OR=1.28]) showed nominal association with this phenotype.

Conclusion: Genetic factors associated with the long-term HIV-controllers without risk of immunologic progression are those previously related to the overall HIV-controller phenotype. The results could help us to understand the biological factors underlying this persistent non-progressor HIV controller phenotype as a crucial issue to consider these subjects as a good model of functional cure and, therefore, for the design of strategies for preventing HIV progression and personalized treatment strategies to those subjects who show CD4+ T-cell loss.

225 lncRNA REGULATES METABOLISM IN MYELOID DENDRITIC CELLS FROM HIV-1 ELITE CONTROLLERS

Jucarita A. Hartana,1 Yelizaveta Rassadkina,1 Ce Gao,1 Enrique Martin-Gayo2, Bruce D. Walker2, Mathias Lichterfeld3, Xu G. Yu4

Background: An HIV-1-specific T cell response is commonly regarded as the backbone of antiretroviral immunity in persons with natural control, while the specific contribution of innate immune cells to such a remarkable disease outcome is less clear. Myeloid dendritic cells (mDCs) are of particular interest, as they play key roles in priming of antiretoviral T cell responses and have the ability to persist long-term in functionally enhanced modification states in ECs.

Methods: Primary mDCs from HIV-1 elite controllers (ECs; n=23) were compared to highly active antiretroviral treated HIV-1-infected patients (HAARTs; n=13) and HIV-1 negative healthy donors (HIVNs; n=61), using RNaseq and CUT&RUN assays to assess the expression of long noncoding RNAs (lncRNAs) and histone modifications, respectively. Seahorse assays were used to analyze metabolic activities of mDCs. LncRNA expression in mDCs was evaluated by PrimeFlow RNA FACs.

Results: LncRNA MIR4435-2HG was upregulated in primary mDCs from ECs compared to HIVNs (P = 5.5e-06) and HAARTs (P = 5.8e-05). Expression of MIR4435-2HG was highly correlated to differentially expressed genes in ECs (n=924) that were enriched in Oxidative Phosphorylation and mTOR/EIF2 metabolic pathways. Upregulation of MIR4435-2HG in ECs was associated with increased oxidative phosphorylation and glycolysis activities, as shown by elevated oxygen consumption rates and extracellular acidification rates in response to TLR3 stimulation using Poly(I:C) in mDCs. Silencing of MIR4435-2HG by siRNA reduced the metabolic states of mDCs. Using the PrimeFlow RNA detection assay, we observed that MIR4435-2HG was expressed in a mDC cell cluster endowed with increase abilities for antigen presentation and previously described as “DC4”, accounting for more than 60% of all mDCs in ECs and <25% in HIVNs (P = 0.0006). Notably, using CUT&RUN assay, we observed significantly increased H3K27ac enrichment at an intronic enhancer region within the RPTOR gene, the main component of mTORC1, in mDCs from ECs compared to HIVNs (P = 0.0286). This genomic locus was specifically predicted to be susceptible to triple-helix formation between chromosomal double-stranded DNA and MIR4435-2HG by the Triplex Domain Finder (TDF) algorithm.

Conclusion: These results suggest a previously unrecognized role of MIR4435-2HG for enhancing the immunometabolic activities of mDCs in ECs through targeted epigenetic modifications of RPTOR, a member of the mTOR signaling pathway.

226 DEFECTIVE FUNCTIONS OF HIV ENVELOPE GLYCOPEPTIDE ASSOCIATE WITH LONG-TERM HIV CONTROL

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Background: The viral envelope glycoprotein complex (Env) is essential in the first stage of HIV infection life cycle and its function has been associated to HIV pathogenesis. Therefore, we explored the relationship between the Env functions and the long-term viral load control in HIV-infected individuals.

Methods: We genotypically and phenotypically characterized 41 Envs from 29 patients with different clinical progression rates. 10 Envs isolated from 7 Long
Term Non Progressors-Elite Controllers (LTNP-EC) individuals with undetectable VL for >20 years and infected in the 90’s; 10 Env clones isolated from 6 viremic LTNP with detectable VL=10,000 copies/ml also infected in the 90’s. As reference, we analyzed 10 ENVs obtained from 6 HIV-1 infected in the 90’s, with both high VL=10^3-5 and a normal chronic infection (Ancient-P) and 11 viral clones from 10 HIV-1 infected individuals between 2013-2014 and with similar VL (Modern-P). We analyzed cell-surface expression levels by flow cytometry; fusogenicity in cell-to-cell fusion assays; CD4 affinity in a viral transfer capacity, which exclusively depends on the binding of gp120 to the CD4 receptor; and infectivity of cell-free viruses in TZM-bl cells.

Results: We did not observe statistically significant differences in cell-surface expression among the four different Env groups. However, a statistically significant lower fusogenicity, CD4 binding and infectivity was observed in Env isolated from LTNP compared to Ancient-P and Modern-P (p<0.05 in all cases). NO differences in these parameters were observed between EC and viremic LTNPs. Genotypic analysis showed differences in the length and glycosylation sites of Env, mainly impacting the V5 loop that showed shorter length in both groups of LTNP. Lower differences were observed in V1-V2 and V4 loops, while V3 loop remained constant among groups. In general, we observed that shorter and less glycosylated Env had lower expression, fusion and transfer capacity.

Conclusion: Our data suggest that there is a link between critical Env-associated viral functions and the clinical progression of the studied individuals. Our data support the hypothesis that poorly functional viral Env could be critical for the control of viral replication and HIV pathogenesis. These viral characteristics represent potential new anti-HIV biomarkers for the development of innovative therapeutic strategies.

227 ELEVATED PLASMA CYTOKINES IN ELITE CONTROL: IP-10 AND MIG PREDICT LOSS OF CONTROL

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Background: Natural control of viral replication in the absence of antiretroviral therapy (ART) occurs in a small fraction of HIV+ individuals. Elite controllers (EC) efficiently control viral blood load under 50 cp/ml while virocontrollers (VC) maintain viral load under 2,000 copies/ml without ART. Distinct miRNA expression has been associated with cell populations in these patients. Hence, we explored the miRNome of EC T cells to identify a miRNA signature related to HIV infection control.

Methods: We performed miRNA-Seq in forty-four samples of CD4 T cells from EC (n=10), VC (n=10), HIV-negative individuals (HIV-; n=4), and typical progressors (TP) (before (NT-TP; n=10) and after ART administration (ART-TP; n=10)). Viral load was monitored for at least more than one year. Reads were aligned to miRBase v21 with Chimira and mirDeep2 and differential expression analysis was carried out between groups (FDR-adjusted q-value<0.1). Target genes were predicted using miRNet and filtered with NCBI’s HIV-host protein interactome, checking for experimentally validated miRNA-gene interactions in DIANA-TarBase 8.0 and mirTarBase v8.

Results: We found four downregulated miRNA in VC/VC vs TP (Fig1): miR-1221-5p (LFC= 0.783), miR-501-3p (LFC= 0.606), miR-99b-5p (LFC= 0.841) and miR-501-3p (LFC= 0.783), that likely regulate 822 host genes.

Conclusion: We observed a specific signature of four downregulated miRNA in ECs and viremic T cells compared to TP. These miRNAs regulating the expression of ErbB2, p53, and CDKN1A may produce the activation of the p53 signaling route, fostering cell cycle arrest and a pro-apoptotic state, as well as the CDKN1A-mediated HIV restriction. The upregulation of p53 in ECs reveals a specific mechanism in ECs, which may modulate pre-integration complex nuclear transport and site-directed integration through TNPO3. Besides, similar miRNA signatures have been found in ECs and HIV-

228 miRNA SIGNATURES IN CD4 T CELLS FROM PATIENTS WITH NATURAL CONTROL OF HIV INFECTION

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Background: Natural control of viral replication in the absence of antiretroviral therapy (ART) occurs in a small fraction of HIV+ individuals. Elite controllers (EC) efficiently control viral blood load under 50 cp/ml while virocontrollers (VC) maintain viral load under 2,000 copies/ml without ART. Distinct miRNA expression has been associated with cell populations in these patients. Hence, we explored the miRNome of EC T cells to identify a miRNA signature related to HIV infection control.

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229 THE VON HIPPEL–LINDAU CULLIN-RING E3 LIGASE REGULATES APOBEC3 CYTIDINE DEAMINASES

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Background: Human apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3; A3) cytidine deaminases can either promote cancer progression or defend against HIV. Nuclear-localized A3s, such as A3B, are...
at low or non-existent levels in primary cells, and often up-regulated in cancer cells causing pathogenic chromosomal mutagenesis. In contrast, increasing T lymphocyte content of cytoplasmic A3G decreases infectivity of progeny VIF-positive HIV virions in vitro. It is not known how A3s are regulated in unaffected cells. Here, we studied the regulation of A3 proteins in unaffected cells.

Methods: Cancer cell lines with endogenous A3 expression and 293T cells expressing HA-tagged A3s were studied. We performed mass spectrometry to identify candidate A3-interacting proteins. Effects of a candidate interactor, the von Hippel–Lindau tumor suppressor (pVHL), on cellular A3 levels, as detected by immunoblotting, were characterized by genetic knockdown, over-expression, and co-immunoprecipitation.

Results: Levels of an endogenous A3 were reproducibly increased by epoxomicin, an irreversible proteasome inhibitor. An A3 interaction with pVHL was identified by a proteomic analysis. siRNA knockdown of pVHL increased levels of an endogenous A3. Ectopic expression of pVHL decreased levels of each A3 (A3A, B, C, D, F, G, Hl and HIII). This decrease was dependent on CRL-pVHL formation, as it was not seen with co-transfection of C6Z2f mutant pVHL which lacks Elongin C binding capacity. The E3 ligase ARIH1 potentiated pVHL-mediated degradation of each A3, except A3H. Finally, CRL-pVHL more effectively degraded nuclear, pro-cancer A3 than the cytoplasmic, anti-retroviral A3G (Figure shows ratio of A3 immunoblot band intensity normalized to Lamin in 11 lysates of 293Ts co-transfected with Flag-pVHL and either HA-A3B or A3G, divided by normalized control HA-A3 intensity in absence of pVHL, P = 0.03, Wilcoxon).

Conclusion: A cellular mechanism of A3 regulation requires formation of CRL-pVHL. pVHL serves as a substrate receptor in that complex, facilitating ubiquitination and proteasomal degradation of target proteins. This parallels the well-characterized mechanism of retroviral VIF in A3 degradation. The observation of better pVHL-mediated degradation of A3B than A3G suggests the possibility of decreasing A3B-accelerated cancer progression without impairing the A3G anti-HIV intrinsic immune defense in people living with HIV (PLWH).

231 IFNL4 rs368234815 SNP IS ASSOCIATED WITH CD4+CD8 RATIO NORMALISATION IN PWH ON ART

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Background: The interferon lambda (IFNL) single nucleotide polymorphism (SNP) rs368234815 has been associated with altered host responses to viral infection. We aimed to determine its impact on CD4:CD8 T-cell ratio normalisation in people with HIV (PWH).

Methods: We analysed T-cell responses in PWH enrolled in the All Ireland Infectious Diseases (AIID) cohort who initiated ART starting January 2001 and had more than one T-cell subset measured at least 6 months later. IFNL4 genotyping was performed on genomic DNA employing allelic discrimination. We collated demographic (age, sex, ethnicity, HIV acquisition risk), clinical and treatment (ART regimens, CD4 and CD8 T-cell counts, HIV RNA data). Primary outcome was time to normalisation of CD4:CD8 ratio (≥1). We used Cox regression models to evaluate associations between ratio normalisation and IFNL4 rs368234815 alleles (ΔG/ΔG or T/ΔG and T/T). Data are median (IQR) unless specified.

Results: 560 PWH, age 34.7 (29.5-40.7) years, 61% male, 53% Caucasian, 48% heterosexual transmission, followed for 7.4 (3.4-11.7) years after ART initiation were included. Nadir CD4 count was 267 (151-415.4) cells/mm³, 39% and 36% were initiated on an NNRTI or PI. Overall 268 (47.8%) achieved a CD4:CD8 ratio ≥1, with median 7.6(95% CI 6.6-9.4) years to ratio normalisation. The probability of CD4:CD8 ≥1 was estimated at 16 (13.0-19.1%), 32 (27.5-35.5%), 38.8 (34.4-42.9%) and 56.5 (51.0-61.3%) at years 1, 3, 5 and 10 respectively. Those with a baseline CD4 ≥350 cells/mm³, were significantly more likely to achieve CD4:CD8 ≥1 compared to those with a T/T major haplotype (HR=2.73, 23.3, 49.0 p<0.001). PWH with baseline CD4 T-cell count ≥350 and expressing IFNL4 rs368234815 (ΔG/ΔG or T/ΔG) were more likely to achieve CD4:CD8 ≥1 compared to those with T/T major haplozygosity (HR=1.51, 95% CI (1.02, 2.23), P=0.038, Figure 1A). There was no association between rs368234815 and CD4:CD8 normalisation in subjects with baseline CD4 <350 cells/mm³ (Figure 1B). The association between rs368234815 (ΔG/ΔG or T/ΔG) and CD4:CD8 normalisation persisted in analyses adjusted for gender, ethnicity, HIV transmission risk and age fitted as either a continuous (HR=1.51, 1.06, 2.38, P=0.024) or fractional polynomial (HR=1.49 (1.00, 2.23), P=0.049) variable.

Conclusion: In PWH starting ART with a CD4 count ≥350, rs368234815 IFNL4 (ΔG/ΔG or T/ΔG) is associated with an increased likelihood of CD4:CD8 ratio normalisation. The role of IFNL4 in modulating persistent inflammation on ART warrants further investigation.
REPLICATED EPIGENETIC ASSOCIATIONS WITH SCD14 AMONG MEN WITH HIV INFECTION

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Background: HIV-infection is characterized by chronic inflammation and immune activation. sCD14 is an important marker for monocyte activation due to microbial translocation and the pathogenesis of HIV. Elevated plasma levels of sCD14 predicts all-cause mortality in people with HIV (PWH). Epigenetic processes, can offer clues into the pathophysiology of inflammatory processes and provide insight for new therapeutic approaches. To date, epigenetic associations with circulating sCD14 levels among PWH are unknown. In this study, we conducted an EWAS of sCD14 levels among PWH and performed replication and meta-analyses to identify epigenetic markers, genes, and pathways linked to sCD14 via DNA methylation changes.

Methods: We conducted an EWAS of sCD14 among 1,074 HIV-positive male participants in the Veterans Aging Cohort Study (VACS) to identify epigenetic signatures of sCD14. Samples were processed at the time of collection. The epigenome-wide DNA methylation levels were measured using the Illumina Infinium Methylation 450K (n=549) and EPIC (850K) BeadChip (n=525). We adjusted for age, race, CD4 count, viral load, body mass index (BMI), hepatitis B and C infection, smoking status, alcohol use and cell type proportions in peripheral blood within each chip type, and conducted meta-analysis to identify significant epigenetic markers.

Results: The mean age of participants was 52.7 years. 84.5% were African American, 10.4% Caucasian, 2.7% Hispanic and 2.4% classified as Other. The average BMI was 25.6 kg/m², and around 79.8% of participants had a smoking history. By surveying more than 300,000 cytosine guanine dinucleotide (CpG) sites of sCD14-association CpG sites (98 sites, 83%) were negatively associated with circulating sCD14 (Bonferroni corrected p-value <0.05) in the meta-analysis. The majority of these sCD14-association CpG sites (98 sites, 83%) were negatively associated with sCD14 levels. All sites had consistent associations between 450K and EPIC chip data (all in the same direction, Pearson correlation R² of 0.91). CpG site of cg00676801 (STAT1) has the most significant association with sCD14 (p-value of 8.43 x 10^-19).

Conclusion: We uncovered novel epigenetic associations with sCD14 in PWH. This was a first step in investigating the relationship between HIV, DNA methylation and inflammatory markers. Further studies are warranted to explore the role of these epigenetic changes in the mechanisms of chronic inflammation and its implications for chronic disease and mortality in PWH.

TH17 CELL MASTER TRANSCRIPTION FACTOR RORC2 REGULATES HIV GENE EXPRESSION AND LATENCY

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Background: Among CD4 + T-cells, Th helper 17 (Th17) cells ensure defenses against bacterial/fungal pathogens at mucosal barriers. During HIV-1 infection, Th17 cells are highly susceptible to infection and depleted from mucosal sites, resulting in mucosal barriers integrity alterations, microbial translocation, systemic inflammation, and disease progression. Additionally, HIV-infected Th17 cells can be long-lived and harbor viral reservoirs (VR) in people living with HIV (PLWH) receiving antiretroviral therapy (ART). Thus, Th17 cells are key players in HIV pathogenesis and VR persistence. Here, we evaluated the role of RORC2, the master regulator of Th17 cell differentiation, on HIV replication and latency.

Methods: Memory CD4+ T-cells were isolated from PBMCs of HIV-uninfected individuals (HIV-), ART-treated (ART+PLWH) and untreated (ART-PLWH) PLWH by negative selection using magnetic beads. Subsequently, cells expressing the Th17 markers RORC2 and CCR6 were isolated by FACs. The NL4.3BL and transmitted/founder THRO HIV-1 strains were used for infections in vitro. A viral outgrowth assay (VOA) was performed with cells from ART+PLWH and ART-PLWH. HIV replication/outgrowth were evaluated by FACS and ELISA. HIV integration was evaluated by nested real-time PCR. RORC2 overexpression was performed in 293T and Jurkat cells infected with VSV-G-pseudotyped HIV-1 LAI env-GFP (HIV-1GFP). For Chromatin immune precipitation (ChIP) experiments, Jurkat cells transduced with a retroviral vector expressing RORC2-myc were infected with HIV-1GFP. Real time PCR signal for HIV LTR NRRE-1 and HIV CS Pol was subsequently evaluated.

Results: The inhibition of RORC2 by tool small molecule decreased HIV replication in CD4 + T-cells in vitro, as well as viral outgrowth from T cells of ART+PLWH and ART-PLWH. Consistently, RORC2 overexpression was higher within HIV-p24+ compared to total T-cells in ART-PLWH upon TCR triggering in vitro.
Moreover, CCR6+ RORC2+ compared to CCR6− RORC2− T-cells of ART+ PLWH were enriched in proviral DNA ex vivo. Furthermore, RORC2 silencing inhibited HIV-1 infection, specifically in CCR6+ T cells, whereas RORC2 overexpression led to enhanced viral replication in cell lines and primary cells. Finally, RORC2 promoted viral gene expression and ChIP revealed that RORC2 binds to the HIV-1 promoter.

Conclusion: Altogether, these results point to RORC2 as a new Th17-specific target for HIV-1 therapy.

234 miR-422a: A Type I Interferon-Regulated Modulator of HIV Replication

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Background: The cytokine interferon-α (IFNα) potently suppresses HIV replication and may accelerate clearance of the latent HIV reservoir. The mechanisms underlying this anti-HIV activity remain to be elucidated. We previously reported that the microRNA (miRNA) miR-422a was the sole miRNA downregulated by IFNα treatment in HIV-infected individuals in vivo, and the extent of miR-422a reduction significantly correlated with viral load reduction. Here, we investigated the molecular basis of this relationship by examining miR-422a effects on HIV replication, latency, and the antiviral capacity of IFNα ex vivo.

Methods: PBMCs were obtained from HIV-infected individuals, and CD4+ T cells were isolated using negative selection. RT-PCR was used to measure miR-422a expression in untreated and anti-CD3/CD28-stimulated CD4+ T cells with or without 1000 units of IFNα. miR-422a overexpression and knockdown Jurkat cell lines were constructed using lentiviral transduction. miR-422a mimic and antagonist were synthesized and transfected into Jurkat cells and primary CD4+ T cells using HiPerfect. Transfected or transduced cells were infected with HIV NL4-3, and HIV replication was measured by p24 ELISA and flow cytometry (Gag). Total RNA-seq was used to evaluate effects of miR-422a overexpression and knockdown on the primary CD4+ T cell transcriptome (N=3 donors). Paired t tests were used to analyze data and FDR was calculated to account for multiple comparisons.

Results: IFNα decreased HIV Gag (p=0.023) and p24 expression (p=0.0028). IFNα downregulated miR-422a in Jurkat (p=0.029) and in primary CD4+ T cells (p=0.001), while HIV infection (p=0.006) or anti-CD3/CD28 stimulation (p=0.0055) upregulated miR-422a in primary CD4+ (p=0.006). miR-422a overexpression increased HIV replication (p=0.014), and miR-422a knockdown inhibited HIV replication (p=0.000023) in primary cells. IFNα-induced HIV suppression was counteracted by miR-422a mimic (p=0.0089) and enhanced by miR-422a antagonist (p=0.0061) in primary cells. RNA-seq data revealed that chromatin and chromosome organization were significantly modulated by miR-422a manipulation (FDR = 0.004).

Conclusion: miR-422a is a key host factor induced by HIV infection and TCR stimulation which supports HIV replication and persistence in the CD4+ T cell compartment via epigenetic effects. Suppression of miR-422a is a critical step underlying IFNα anti-HIV activity. Our data suggest that pharmacologic manipulation of miR-422a should be explored as an HIV cure strategy.

235 Gut Ox40+ CD4+ T Cells Strongly Correlate with Markers of Progression in Treated HIV

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Background: Ox40+ T cells are a natural reservoir of HIV in patients treated with ART. However, the role of these cells in HIV progression is not clear.

Methods: PBMCs were obtained from treated patients with or without HIV-1 replication. Ox40+ CD4+ T cells were isolated using negative selection. Flow cytometry was used to determine the proportion of Ox40+ CD4+ T cells and the proportion of Ki67+ CD4+ T cells. Spearman’s rank test was used to analyze the correlation between the proportion of Ox40+ CD4+ T cells and the proportion of Ki67+ CD4+ T cells.

Results: The proportion of Ox40+ CD4+ T cells was significantly correlated with the proportion of Ki67+ CD4+ T cells (p<0.001). The proportion of Ox40+ CD4+ T cells was also significantly correlated with the proportion of Ki67+ CD4+ T cells (p<0.001) in patients with HIV-1 replication.

Conclusion: Ox40+ CD4+ T cells are a natural reservoir of HIV in patients treated with ART. The proportion of Ox40+ CD4+ T cells is significantly correlated with the proportion of Ki67+ CD4+ T cells, suggesting that these cells may play a role in HIV progression.
THE COMPLEMENT PATHWAY IS ACTIVATED IN HIV AND ASSOCIATED WITH NON-AIDS COMORBIDITY

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Background: Effective antiretroviral therapy (ART) has extended the lifespan of people with HIV (PWH), who increasingly experience non-AIDS comorbidities which affect their overall health and contribute to early mortality. Occurrence of these comorbidities has been linked to elevated levels of systemic inflammation. Numerous clinical biomarkers including CRP, IL-6, and sCD14 have been shown to be independently associated both with comorbidity prevalence and early mortality. The precise immune pathways involved in the pathogenesis of these inflammatory non-infectious complications, however, remain poorly understood hampering targeted interventions. We hypothesized that unbiased serum proteomics would identify novel pathways enriched in PWH and associated with non-AIDS comorbidities.

Methods: Single time point plasma samples from a subset of participants of the AGEnHIV Cohort Study were subjected to aptamer-based proteomic screens (SOMAscan), yielding quantification of 1,317 serum proteins. A total of 78 participants were selected including 51 people with HIV (PWH) and 27 seronegative controls (HC) matched for sex/sexual behaviour, age, BMI, and smoking status. Comorbidities captured included non-AIDS defining cancers, myocardial infarction, angina pectoris, ischemic stroke/transient ischemic attack, peripheral arterial disease, heart failure, chronic obstructive pulmonary disease, type 2 diabetes, advanced liver fibrosis, osteoporotic fracture, and renal insufficiency.

Results: The median age for both PWH and HC was 55.3 years and 33% of participants were women. The median CD4 count in PWH was 815 cells/μL and all were virologically suppressed. We found that complement activation was the most enriched pathway in the serum proteome of PWH by two independent pathway classification databases (WikiPathways and Human Phenotype Ontology). Complement component C5 was among the top most enriched specific proteins in PWH and correlated significantly with having experienced a clinical non-AIDS event among PWH. C5 retained its significant association with comorbidity prevalence after adjustment for IL-6, D-dimer, suPAR, and sCD14 levels (P=0.043, OR per 1 IQR = 1.73), and did not correlate with these marker levels.

Conclusion: We propose that complement pathway components like C5 should be further evaluated as predictive biomarkers for HIV-associated non-infectious comorbidities. If validated, they may serve as more informative endpoints in HIV-1 control and disease progression.

DIGITAL SPATIAL PROFILING OF FIBROCYTIC LYMPH NODE MICROENVIRONMENTS IN CHRONIC HIV

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Background: Characterizing the cellular microenvironment of fibrotic regions in lymph nodes (LN) may further our understanding of LN fibrosis during chronic HIV disease.

Methods: Inguinal LN excisions were done by a board-certified surgeon on 6 virally-suppressed persons living with HIV (PLWH) on ART and 4 HIV-uninfected participants (controls) recruited at the University Clinics at Kaka‘ako at the John A. Burns School of Medicine. A portion of each LN was formalin-fixed, paraffin-embedded (FFPE) and slide-mounted tissue sections were prepared. H&E staining was performed on LN slides and fibrotic regions were quantified by a board-certified pathologist. Digital spatial profiling (DSP) was performed on LN slides (NanoString Inc.) using the GeoMx DSP platform. LN slides were stained with fluorescent antibodies targeting CD3, CD8, and CD68 to identify cells of interest (CDI); CD8 and CD4 T cells and macrophages (Mφ). Two fibrotic and two non-fibrotic regions were identified for each LN slide remotely by a board-certified pathologist and protein expression profiling for each COI in each region was performed using nCounter barcoding technology. Statistical analyses performed were T-tests and Linear Mix Model tests.

Results: LNs excised from PLWH had slightly higher amounts of fibrosis as compared to controls (HIV+ vs. HIV- median LN fibrosis: 25% vs. 18%), however not statistically different (p=0.511). Although we did not observe significant fold change (log2FC) differences in protein expression profiles in each LN COI between PLWH and controls, we did find significant log2FC in protein expression profiles in each LN COI between fibrotic and non-fibrotic regions in both groups. In PLWH: Mφ in fibrotic LN regions had a higher protein profile of CD14 (log2FC=1.035; p=0.001, FDR=0.037), B7-H3 (log2FC=0.728; p=0.012, FDR=0.119), α-SMA (log2FC=0.439; p=0.035, FDR=0.216), and CD163 (log2FC=1.372; p=0.045, FDR=0.233); CD8 T cells had higher OX40L (log2FC=0.905; p=0.002, FDR=0.099), fibronectin (log2FC=1.247; p=0.006, FDR=0.020), and CD34 (log2FC=1.026; p=0.009, FDR=0.027); CD4 T cells had higher CD34 (log2FC=1.047; p=0.005, FDR=0.033), fibronectin (log2FC=1.275; p=0.005, FDR=0.033), OX40L (log2FC=0.880; p=0.009, FDR=0.034) and B7-H3 (log2FC=0.444; p=0.044, FDR=0.104). Controls had similar increases in protein profiles for each COI in fibrotic regions.

Conclusion: DSP analyses reveal distinct immune cell protein expression profiles in fibrotic LN microenvironments, which may inform targeted fibrosis treatments.

EFFECT OF HIV ACQUISITION ON SYSTEMIC INFLAMMATION IN A SEROCONVERSION COHORT

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Background: Despite effective antiretroviral therapy (ART), systemic inflammation is reported in people living with HIV (PLWH) compared HIV-uninfected controls. This has been attributed to residual HIV replication, bacterial translocation, coinfections, and side effects of ART.

Methods: The Sabes study followed 2109 uninfected high-risk Peruvian men-who-have-sex-with-men and transgender women with monthly HIV testing by serology and PCR. Participants with incident HIV were randomized to initiate ART immediately or to defer for 24 weeks. Biomarkers (sCD14, sCD163, SuPAR, IFNα2, IFN-γ, IL-1β, IL-6, IL-8, IL-10, IP-10, Leptin, MCP-1, TNF-α, LBP, CRP) were quantitated in plasma at two time points before and two after infection and ART-suppression (>6 months and >12 months after suppression). Stability of each biomarker across the two pre- and the two post-infection/ART-suppression timepoints were assessed across all participants, by immediate vs. deferred ART arms and by individual; with significance (p<0.05) calculated.

Results: Biomarkers were determined for 50 participants (21 randomized to initiate ART immediately and 29 at 24 weeks; with 3 initiating earlier when they met CD4 thresholds for ART in local treatment guidelines). Across the 2 pre-infection timepoints 3 of 15 biomarkers (IP-10, IL-6, CD163) significantly varied, while the others were at steady state. Compared to the pre-infection
values, at the first post-infection timepoint, Leptin (n=9/50) and LBP (n=4) decreased and MCP-1 (n=6) increased; with changes in Leptin and MCP-1 observed in both immediate and deferred ART-initiation arms. Comparing levels during ART-suppression to pre-infection timepoints, IFN-γ and LBP decreased in the immediate arm, while CRP increased in the deferred arm. During ART-suppression, Leptin and LBP increased and IL-8 decreased in both arms over time. Considering each arm separately, IL-8 decreased, and Leptin significantly increased during ART-suppression for the deferred arm but not for the immediate arm. A sustained increase in ≥2 pro-inflammatory biomarkers during ART-suppression was not observed in any of the 50 participants.

Conclusion: This longitudinal study of PLWH who initiated ART during early infection found little evidence for sustained elevations in pro-inflammatory biomarkers attributable to HIV infection. Instead, elevations of one or a few biomarkers were detected in a minority of participants following infection; biomarkers from before and after infection were stable in most participants.

240 mTOR ACTIVATION LIMITS LPS-INDUCED MONOCYTE INFLAMMATORY AND PROCOAGULANT RESPONSES

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Background: Microbial translocation and subsequent lipopolysaccharide (LPS) activation of monocytes via TLR4 is likely to increase cardiovascular disease (CVD) risk in persons living with HIV. LPS induces metabolic signaling in monocytes necessary for the production of inflammatory cytokines and a procoagulant, tissue factor (TF). Using primary monocytes, we tested the hypothesis that LPS-induced pro-inflammatory and -coagulant responses are supported by mTOR activity and contribute to CVD risk. Paradoxically, metabolomics analyses here demonstrate that mTOR activates a metabolic pathway that limits abundance of these gene products in monocytes.

Methods: Human primary monocytes were treated with LPS in the presence or absence of catalytic mTOR inhibitors (mTORi) and compared with untreated monocytes. Samples were analyzed using both RNAseq and metabolic profiling. Changes in cytokine production were determined by ELISA and intracellular flow cytometry. Phenotypic changes in monocyte activation status and TF expression were also monitored using flow cytometry.

Results: Transcriptomic analysis revealed that treatment of primary human monocytes with mTORi potentiates both LPS-induced pro-inflammatory cytokines (IL-1β and IL-6) and coagulation-mediating TF. We found NF-κB-driven transcriptional activity enhances expression of F3 (TF) with LPS stimulation. Using primary monocytes, we tested the hypothesis that LPS-induced pro-inflammatory and -coagulant responses are supported by mTOR activity and contribute to CVD risk. Paradoxically, metabolomics analyses here demonstrate that mTOR activates a metabolic pathway that limits abundance of these gene products in monocytes.

Conclusion: Collectively, our data support the hypothesis that mTOR signaling checks potentially harmful responses in pro-inflammatory monocytes. Thus, our results are relevant for understanding metabolism-related mechanisms of accelerated pro-inflammatory conditions in PLWH. They also are significant for SARS-CoV-2 infection, which also impairs the gut barrier, depletes NAD+ pools, and causes coagulopathy. This suggests that the LPS- and mTOR-related mechanisms defined here warrant investigation in SARS-CoV-2 cytokine storm-induced pathogenesis.

241 MONOCYTE SUBSETS AFTER LONG-TERM ART AND MEASURES OF HIV PERSISTENCE IN ACTG AS321

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Background: Several studies have investigated the role of monocytes in HIV infection, specifically in HIV-associated chronic inflammation. However, it is unclear how these immune cells correlate with measures of HIV persistence in individuals on long-term suppressive ART.

Methods: In this cross-sectional study, we determined the frequencies of classical CD14+CD16-), intermediate (CD14+CD16+), and non-classical (CD14dimCD16+) monocytes in ACTG AS321 participants at study entry using flow cytometry. We also obtained plasma levels of pro- and anti-inflammatory markers by ELISA and multiplex assays. We measured levels of residual plasma HIV RNA and, in CD4+ T cells, HIV DNA, cell-associated HIV (CAR), and intact proviral DNA (IPD) by PCR assays. Spearman correlations were used to assess associations between continuous measures and were adjusted for age, sex, pre-ART RNA and CD4+ T cell count, and years on ART.

Results: Participants (N=225) had median age of 49 years, median pre-ART CD4 and CD8 at study entry of 255 and 681 cells/μL, respectively, median pre-ART HIV RNA of 4.6 log10 copies/mL, and median of 7 years on suppressive ART. Median frequency of classical monocytes was 84.6% (IQR 79.1, 88), intermediate monocytes was 1.4% (0.9, 2.6), and non-classical classical monocytes was 1.3% (0.6, 2.3). After adjusting for potential confounders, none of the monocyte subset frequencies correlated with HIV DNA, CAR, or residual plasma HIV RNA (-0.08 ≤ r ≤ 0.08). Similarly, frequencies of the subsets were not associated with IPD (N=24). Frequencies of classical monocytes modestly correlated with plasma levels of pro-inflammatory markers IL-6 (r=0.17, p=0.01), CCL2 (r=0.30, p=0.04), and sCD163 (0.16, p=0.02), and negatively correlated with years on ART (r=-0.18, p=0.01). Higher levels of intermediate monocytes correlated with higher levels IP-10 (r=0.14, p=0.03) and sCD163 (r=0.14, p=0.04) and showed a trend for negative correlation with anti-inflammatory IL-10 levels (r=-0.26, p=0.07). None of the immunologic parameters correlated with frequencies of non-classical monocytes.

Conclusion: In this study of virally suppressed people with HIV on long-term ART, monocyte subsets were not associated with measured markers of HIV persistence. Classical and intermediate monocytes had modest associations with levels of inflammation and immune activation. Further studies are needed to define the role that monocyte subsets play in HIV persistence and in inflammation and immune activation.
242 IMMUNO-INFLAMMATORY PROFILE OF ADVANCED-HIV–INFECTED PERSONS IN A COVID-19 OUTBREAK

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Background: It is unclear if chronic immune dysfunction in HIV might affect immune response in COVID19. Our aim was to analyze the inflammatory profile and the immune response to COVID19 of a cohort of patients (pts) with a previous AIDS diagnosis and SARS-CoV-2 infection in an assisted living facility in which an outbreak occurred, and to compare them to HIV-negative COVID-19 patients and advanced HIV-positive without COVID-19.

Methods: Levels of the inflammatory markers (IL1, IL6, IL8 and TNFα) were analyzed in advanced HIV+ pts without COVID-19 (group 1), in advanced HIV+ pts infected by SARS-CoV-2 (group 2) along with SARS-CoV-2-specific T-cell response, and in HIV- pts with mild/moderate COVID-19 consecutively hospitalized during the first pandemic wave (group 3). Inflammatory cytokines were quantified by automatic ELISA assay (ELLA system); antibodies titer was evaluated by ELisa assay (Disease) and SARS-CoV-2 specific T cell response was quantified by Elispot assay. Mann Whitney was used for comparison between each couple of groups.

Results: The analysis included group 1 (n=76 pts), group 2 (n=30), group 3 (n=58). Pts of group 1 and 2 did not differ by age, gender and duration of HIV infection. Median CD4 and CD8 was higher in group 2 vs group 1 (348/mm3, vs 118/mm3 and 756 vs 518; p<0.01). HIVRNA was <50cps/ml in 96.7% of pts in group 2 and 70% in group 1. HIV+ /COVID19 pts had lower prevalence of COVID19 symptoms than HIV-uninfected COVID19 patients (p<0.0001) (Figure 1A). Pneumonia was diagnosed in 66% of pts in group 2 and 86% in group 3 (p=0.141), and there was no difference for SpO2 at COVID19 diagnosis (p=0.146). 10% of pts in group 2 and 15% in group 3 died during follow-up (p=0.475). Of note, we observed significant higher level of IL6, IL8 and TNFα in group 3 vs group 2 (p<0.05) and group 1 (p<0.001) (Figure 1). The median time to SARS-CoV-2 clearance was 18 (IQR 16-25) days in group 2, and 12 (IQR 6-23) days in group 3 (p=0.002).

Conclusion: These preliminary results suggest that HIV infection, even in advanced stage, did not seem to negatively impact on COVID-19-related inflammatory state. Moreover, specific immune response in these patients did not differ than that observed in HIV-negative COVID-19 pts. Further investigations are needed to better define the interplay between HIV and SARS-CoV-2.

243 mTOR REGULATION OF ANTIGEN-SPECIFIC CD4+ T-CELL RESPONSES IN MYCOBACTERIAL IRIS

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Background: Immune reconstitution inflammatory syndrome (IRIS) is an aberrant inflammatory complication observed in HIV- individuals following the initiation of antiretroviral therapy (ART) frequently associated with mycobacterial co-infections. IRIS is characterized by dysregulated antigen specific CD4+ T cell responses against the opportunistic pathogen. T cell activation is dependent on metabolic reprogramming and the central cell metabolism pathway, mammalian target of rapamycin (mTOR), can regulate T cell effector function. We hypothesized that mTOR activation is associated with the robust antigen specific CD4+ T cell responses in IRIS and the mTOR pathway could be targeted for therapeutic measures.

Methods: Cryopreserved PBMCs at the IRIS event or equivalent time point after ART from 6 HIV+ patients with mycobacterial-IRIS (mycobacterium tuberculosis [MTB], M. avium complex [MAC], and M. marinum) and 6 HIV+ patients with mycobacterial infection (MTB and MAC) who did not develop IRIS were stimulated for 16 hours with mycobacterial antigen (PPD and heat inactivated MAC). mTORC1 inhibitor rapamycin (20-500ng/ml) was added at the beginning of stimulation. Intracellular IFN-γ and mTOR downstream target phosphorylated S6 (pS6) were detected using flow cytometry after stimulation.

Results: Under unstimulated condition, pS6 expression in CD4+ T cells was low with no difference between IRIS, no-IRIS, and healthy control groups (Figure 1A). Following mycobacterial antigen stimulation, IFN-γ production and pS6 level were significantly increased (p<0.0001) in CD4+ T cells in HIV+ patients with mycobacterial co-infection (Figure 1B, C). Rapamycin reduced pS6 expression and IFN-γ production in CD4+ T cells in a concentration independent manner (Figure 1D, E). When comparing IRIS with no-IRIS groups, IFN-γ producing CD4+ T cells in all IRIS patients were significantly decreased (p=0.031) in the presence of low concentrations of rapamycin at 20-50ng/ml (Figure 1F) reaching plateau at higher concentrations. The effect of rapamycin on IFN-γ production in CD4+ T cells after stimulation was not statistically significant in HIV+ no-IRIS patients (Figure 1F).

Conclusion: Increased mTOR activity was observed in CD4+ T cell following antigen stimulation in HIV+ patients with mycobacterial co-infection. Rapamycin can successfully reduce pS6 levels and IFN-γ production in CD4+ T cells from patients with IRIS following antigen stimulation, which highlights the potential of mTOR pathway as a therapeutic target for IRIS.

IMPACT OF FATTY ACIDS ON T-CELL FUNCTION AMONG PEOPLE LIVING WITH HIV

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Background: HIV-infected patients have increased incidence of metabolic disorders with aberrant lipid profiles, due to both HIV itself as well as antiretroviral treatment. The contribution of lipid exposure on the function of T cells in HIV has not been explored.

Methods: Measurement of metabolic parameters including lipid and fatty acid (FA) stores, FA transporter (CD36) levels, and T cell inhibitory receptor expression was performed by flow cytometry among HIV-infected (n=35) and uninfected (n=5) subjects. The impact of FA exposure (oleic or linoleic acid) with or without CD36 inhibitor, sufinol-1-succinimidyl oleate (SSO), was measured after stimulation of PBMCs with staphylococcal enterotoxin B (SEB).

Results: HIV-infected subjects had higher expression of CD36 on CD8+ T cells compared to healthy individuals (53.4% vs 26%, p=0.03). In HIV-infected subjects, among CD8+CD36+ T cells, the majority were effector memory (37.4%), followed by naive (20.0%), TEMRA (19.9%) and central memory (12.9%) subsets (p<0.001 for all comparisons). CD8+CD36+ T cells have higher expression of immune inhibitory receptors PD-1 (26% vs 15.2%) and CD244 (37.5% vs 23.9%) compared to CD8-CD36+ cells.
had higher levels of FA stores (C12 and C16) compared to CD36- cells (p=0.01 and p=0.03), but comparable neutral lipid stores. Upon exposure to oleic acid, CD6+ T cells demonstrated 96.4±1.5% and 98.0±2.1% reduction in TNF-α and INFγ production in response to SEB stimulation, compared to cells not exposed oleic acid (p<0.01 for both). Though exposure to fatty acids almost completely eliminated cytokine production, exposure to 500 (CD36 inhibitor) restored TNF-α and INFγ production to 84.5±5.8% and 86.5±5.2% of baseline levels respectively. Linoleic acid exposure produced similar results to oleic acid.

Conclusion: These data suggest that CD36-mediated FA transport may contribute to T cell dysfunction and compromise function of CD8+ T cells in HIV infection. Future studies to elucidate altered fatty acid metabolism, fatty acid transport, and signaling pathways are needed to provide insight on T cell exhaustion in HIV infection, and clinical impact in non-AIDS related conditions.

245 EFFECT OF HIV ON THE DISTRIBUTION OF NK CELL SUBSETS AND THEIR PHENOTYPE IN INFANTS

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Background: NK cells during early life exhibit a distinct phenotype and function that may impact their ability to control HIV infection. Previous studies in perinatally HIV infected children showed alterations in the NK cell compartment when compared to HIV Exposed and Unexposed Uninfected children. This study was performed to investigate the effect of HIV infection on the phenotype of NK cells in neonates prior to ART initiation and its association with Pre-ART plasma HIV viral load (VL).

Methods: In a cohort of 33 untreated HIV Infected (HIV) and 35 HIV Exposed Uninfected (HEU) infants (age range 1-2months) from Maputo (Mozambique), we performed in depth phenotypic analysis using a 28 color flow cytometry panel to identify NK cell subsets based on CD56 and CD16 expression such as the cytokine producers (CD56++CD16-, CyP) and cytotoxic (CD56+CD16+, CTX).

NK subsets were further analyzed for the expression of markers of immune activation (IA), immune regulation and trafficking, and compared between HIV and HEU by Mann-Whitney t-test and correlated with VL in HIV by Pearson correlation test.

Results: HIV showed an altered distribution of NK cell subsets with a lower frequency of CyP NK (p<0.001) and higher frequency of CTX NK (p<0.05) compared to HEU. However, IA of these subsets was increased in HIV as demonstrated by the higher frequency and MFI of the IA marker CD38 and chemokine receptorCCR5 (p<0.05). Moreover, the frequency of CD56+ CTX NK cells and MFI of CCR5 in CyP NK cells showed a positive association with VL (p=0.05, r=0.4). Frequency of circulating follicular helper cells (Th9, CD27+CD45R0+CXCR5+ μl) was lower in HIV (p<0.05) but proportions of Th9 expressing CD38, HL-DR, CD8, ICOS and TIGIT were greater. Notably, within the naive CD4 T cells, HIV showed a different distribution of CD31 compared to HEU with higher proportion of CD31+ and lower CD31-.

Conclusion: The data demonstrate that HIV infection induces a generalized immune activation in both CD4 and CD8 T cells and in their maturational subsets within the first 2 months of life prior to ART initiation. Circulating Th9 and Naïve CD31+ CD4+ were the most affected subset by HIV infection in infants which impact reservoir establishment and HIV persistence.

247 T-CELL IMMUNE DYSREGULATION AND MORTALITY IN WOMEN WITH HIV

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Background: Dysregulation of adaptive immunity is a hallmark of HIV infection that persists even on antiretroviral therapy (ART), and may contribute to higher risk of non-HIV-related diseases in people with HIV compared to people without HIV. Yet few large, prospective studies with long follow-up have examined associations of adaptive immunity deficits with morbidity in HIV.

Methods: Multiparameter flow cytometry was applied to peripheral blood mononuclear cells from 612 women with HIV in the Women’s Interagency HIV Study (WIHS). Samples were collected from 2002-2005, and underlying cause of death ascertained from the National Death Index up to 2018. Using competing risk regression, we examined associations of adaptive immunity deficits with mortality in HIV.

Results: At baseline, participants ranged in age from 35 to 47 (median: 41) and 67% were on ART. Among 104 deaths during median 13.3 years follow-up, 90 were due to natural causes (53 non-HIV-related, 37 HIV-related), 10 to external causes, and 4 were missing cause. Higher activation, senescence, and exhaustion of CD4+ T-cells, activation of CD8+ T-cells, and lower co-stimulation of CD4+ T-cells, were significantly associated with natural cause mortality, adjusting for age, demographic, and behavioral characteristics, cardiometabolic factors, and HIV-related factors.

Conclusion: Distinct features of the developing infant immune system may impact the establishment of reservoir, as well as the potential for its elimination. In this regard, naive CD4 T cells were recently suggested as an important CD4 T cell subset for HIV infection and persistence in infants as they account for almost 90% of the whole CD4 T cell population. In this study, we aimed to characterize the phenotypic differences of T cells in a cohort of HIV perinatally infected infants after ART initiation to understand the immune milieu in which the HIV reservoir is established.

Methods: In a cohort of 33 HIV perinatally infected (HIV) and 35 HIV Exposed Uninfected (HEU) infants (age range 1-2months) from Maputo (Mozambique), we performed in depth immune phenotypic analysis using a 28 color flow panel to evaluate immune activation and immune exhaustion markers in CD4 and CD8 T cells and maturation subsets before ART initiation. Additionally, naive CD4 T cells were divided into CD31+ and CD31- subsets, a marker for recent thymic emigrants and null/low proliferation, respectively.

Results: Immune Activated (HLA-DR+CD38+) CD4 and CD8 T cells were higher (p<0.001 and p<0.0001 respectively) in HIV infants compared to HEU but no correlation with pre-ART viral load (VL) was observed. The frequency of PD-1 on total CD8 was higher (p<0.0001) in HIV, and a positive association with pre-ART VL was found (p=0.05, r=0.4). Frequency of circulating follicular helper cells (Th9, CD27+CD45R0+ CXCR5+) was lower in HIV (p<0.05) but proportions of Th9 expressing CD38, HL-DR, CD8, ICOS and TIGIT were greater. Notably, within the naive CD4 T cells, HIV showed a different distribution of CD31 compared to HEU with higher proportion of CD31+ and lower CD31-. Moreover, these 2 subsets were affected differently by HIV infection with CD31+ Naïve showing higher expression of activation markers (CD25, HLA-DR and PD-1) when compared to HEU, while no differences were observed in CD31- Naïve CD4 T cells.

Conclusion: The data demonstrate that HIV infection induces a generalized immune activation in both CD4 and CD8 T cells and in their maturational subsets within the first 2 months of life prior to ART initiation. Circulating Th9 and Naïve CD31+ CD4+ were the most affected subset by HIV infection in infants which impact reservoir establishment and HIV persistence.
CD4+ T-cells were marginally associated with the outcome, and these were not attenuated by further adjustment. Associations of CD4+ T-cell activation, exhaustion, and co-stimulation with natural cause mortality tended to be stronger in women with uncontrolled HIV (detectable viral load or CD4 cell count <500 cells/mm³) vs. controlled HIV, though interactions were not significant.

**Conclusion:** Consistent with HIV pathogenesis, dysregulation in multiple CD4+ T-cell subsets is associated with HIV-related mortality. Activation and exhaustion of CD4+ T-cells may also be involved in non-HIV-related mortality, independent of cardiometabolic or HIV-related factors.

**Figure 1.** Adaptive immune markers and mortality in women with HIV. Competing risk regression was used to assess the associations of CD4+ and CD8+ T-cell activation (%CD38+HLADR+), senescence (%CD57+CD8+), exhaustion (%PD-1+), and co-stimulation (%CD57+CD28+) with natural cause mortality (i.e., excluding external causes), HIV-related mortality, and non-HIV-related mortality. Immune markers were Z-score standardized; plot shows hazard ratios (95% CI) for one standard deviation increase in the immune markers. Model 1 adjusts for age, study site, race/ethnicity, income, education, crack/cocaine use, injection drug use, alcohol use, smoking, and hepatitis C virus infection. Model 2 adjusts for all Model 1 covariates and BMI, systolic blood pressure, cholesterol, HDL, antihypertension medication, lipid lowering medication, and diabetes. Model 3 adjusts for all Model 2 covariates and CD4 cell count, HIV viral load, antiretroviral therapy, and AIDS.

**248 IMPACT OF REPRODUCTIVE AGING ON IMMUNE FUNCTION IN CISGENDER MEN AND WOMEN WITH HIV**

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**Background:** Women represent more than half of all HIV infections. Understanding the changes in the immune system during reproductive aging is crucial to inform treatment and care strategies.

**Methods:** Longitudinal samples (N=415) from virally suppressed cis-gendered women (N=61) and men (N=31) were retrospectively identified from the AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT) population. Participants were aged 40-55 at the time of ART initiation and virally suppressed (<20 copies/ml) throughout the study period. Participants did not report taking hormonal therapy during follow-up. Cryopreserved peripheral blood mononuclear cells were thawed and stained with a panel of fluorescence-tagged antibodies to identify cell populations of interest. Flow cytometric analysis included initial gating for singlet events and cell viability, followed by captured event clustering with the R software package, Phenograph. Immune phenotypes were assigned using a computational algorithm and confirmed manually. Clusters were analyzed longitudinally for significant variability across participants by sex using a linear mixed-effects (LME) model.

**Results:** We identified 327 immune cell clusters including CD4+ (N=64), CD8+ (N=57), double negative CD4-CD8- (N=13), and double positive CD4+CD8+ (N=12) T cells. The remaining 181 clusters remained undefined based on the immune markers included in the panel. LME models identified 54 clusters that significantly differ by sex (median follow up 7.7 years [95%I: 2.3-15.1]). The most dynamic T cell cluster was a phenotypic mixture of naïve and central memory with high CD45RA and bimodal FoxP3 expression. In this case, the subset increased significantly over time in men, but not in women. Men also experienced a faster decline in double negative (CD4-CD8-) T cell clusters and double positive T cell clusters.

**Conclusion:** Women have increasing exhausted T cells as they age, whereas men have an increasing population of regulatory T cells. These changes in immune cell phenotype may be linked to comorbidities associated with sex-specific patterns of inflammaging and HIV persistence.

**Figure Legend:** Two PBMC clusters were identified by a linear mixed-effects model as being the most dynamic between men and women as they age (A). The proportion of cells in the cluster from each participant is plotted against the age of the participant at the time of sampling (cluster 45 on left, cluster 9 on right). Solid lines show fit lines and shaded regions 95% confidence intervals for men (blue) and women (red) as participants age. In an effort to identify the type of cells in the cluster, distribution curves of the marker expression in all events analyzed (B, green) were compared to the distribution for that marker in each cluster (B, orange). Vertical dashed lines represent the mean expression of a particular marker for all events analyzed. Phenotypes were inferred by calling markers “positive” or “negative” based on the distribution of marker intensity within a cluster compared to the overall mean line. Cluster 45 is shown above cluster 9.

**249 EXOSOMES AND VIRUSES: A TALE OF 2 OVERLAPPING WORLDS**

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**Background:** Extracellular vesicles (EVs) play a significant role in intercellular communication by serving as a carrier for the transfer of membrane and cytosolic proteins, lipids, and RNA between cells.

**Methods:** In recent years, using state of art technologies such as RNA seq, RT-PCR, and single cell omics, we have found that virally infected cells including HIV-1, HTLV-1, Rift Valley Fever, Zika, Ebola, and Coronavirus infected cells secret exosomes that contain biomarker of these infections in urine, saliva, CSF, and blood. We have been able to separate and characterize EVs from several different viruses including HIV-1.

**Results:** These EVs are not infectious and have a different density than infectious virions using gradients. They contain various viral RNA including TAR (non-coding RNA), Nef, gp120/160 and Tat. The origin of these EVs are secreted from virally infected cells, especially when treated with cART or Interferons. They are present in patient samples tested (plasma and CSF, 33%-95%) to date (4 cohorts of 5-20 patients each). The EVs contribute to pro-inflammatory signals
in the naïve recipient cells using TLR3 signaling. Recently, we have asked about the timing difference between EV and virus release from infected cells using serum starvation experiments from cells followed by release. Results from supernatants of uninfected cells showed a peak of tetraspanin proteins (CD63, CD81, and CD6) at 6 hours and a gradual decrease of all EV associated proteins by 24 hours. However, the EV from HIV-1 infected cells showed all three tetraspanins present at 3 hours and expression gradually increased up to 24 hours. HIV-1 viral proteins (p24, gp120, Nef) expression was present at 6 hours and continued to increase and peaked at 24 hours. HIV-1 supernatant 6-hour sample was found not to be infectious. However, infectious HIV-1 was successfully rescued from 24-hour sample.

Conclusion: Our data indicates that EV release may occur prior to viral release in infected cells, thereby implicating a potentially significant effect of EVs on uninfected recipient cells prior to subsequent viral infection.

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250 STRAIN-DEPENDENT EFFECTS OF SIGNAL PEPTIDES ON HIV-1 Env GLYCOSYLATION AND FUNCTIONS

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Background: HIV-1 Env glycoprotein is a trimer of heterodimeric gp120 and gp41 subunits that are produced from a gp160 precursor. The N terminus of gp160 contains a signal peptide (SP) which is essential for targeting the nascent protein to the endoplasmic reticulum where the Env SP is cleaved and removed from the maturing Env. Thus, the Env SP is not a part of the mature Env present on the surfaces of virions and infected cells. Nonetheless, similar to the mature Env, the Env SP displays an extraordinary genetic diversity, although its significance is not well understood. This study investigated the influence of SP sequence diversity on Env glycosylation and functions.

Methods: We constructed chimeric infectious molecular clones by swapping the native SPs of HIV-1 isolates CMU06 and SF162 with SPs from other HIV-1 isolates (MW965.26, 398F1, CH119, and 271.1). SP-swapped and WT viruses were constructed in H9 cells and evaluated for site-specific glycan contents by mass spectrometry. We also assessed the effects of SP exchanges on DC-SIGN-mediated virus transmission and virus neutralization by monoclonal antibodies (mAbs).

Results: Comparison of virion-derived total Env from SP-swapped viruses vs their respective WT revealed strain-specific alterations in the proportions of oligomannose and complex glycans at many glycosites particularly at the trimer apex and base. Modified glycan compositions were associated with reduced DC-SIGN-mediated transmission of CMU06 but not SF162. Differential effects were also seen on CMU06 vs SF162 sensitivity to neutralization by mAbs targeting different epitopes, including YV12, V3 and gp41.

Conclusion: These data demonstrate the contribution of SP in determining Env glycosylation, virus transmission and antibody recognition in a virus strain-specific manner. Hence, this study provides direct evidence for a critical role of the Env SP in virus-host interaction.

251 AN EPIGENETIC ARCHITECTURE THEORY TO ASSESS THE FITNESS OF VIRAL SEQUENCES

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Background: Intrahost viral dynamics are an underlying cause of transmissibility. Architectural studies of viral sequences may retrieve epigenetic information to assess virulence. Nucleotide statistics of a viral sequence — e.g. the proportions of the CpG duplet and the Guanine-Cytosine content (GC) — are ‘architectural traits’, sequence-independent, and can be achieved similarly by independent genetic lineages. Among RNA viruses, epigenetic interactions have been reported between viral genomic Gc6 contents and immune targeting enzymes of the host cell, affecting viral replication. In turn, GC content has been suggested to affect gene expression and evolution rates. We propose that these architectural traits can be coordinates of an n-dimensional space, and space occupancy could predict disease progression measures, such as viral load (VL) and CD4.

Methods: HIV pol sequences obtained for clinical and surveillance purposes in Mexico, Guatemala, Belize, El Salvador, Honduras and Nicaragua between 2014 and 2019 were included. To visualize the effect of architectural occupancy — i.e. space occupancy of viral loads on the CpG/GC plane — sequences were plotted over an ‘Architectural Space’ (AS) with two axes: CpG vs. GC. Then, each point was colored and sized according to its associated VL. Higher associated VLs were expected to occupy the bottom-left sections of the AS (i.e. lower CpG and GC). Also, two linear models were built: i) log₁₀(VL) ~ %CpG + %GC; and ii) and CD4 ~ %CpG + %GC. Slopes were expected negative for both predictors versus VL and, positive versus CD4.

Results: We analyzed 4027 HIV-pol sequences. %CpG ranged from 0.01 – 0.7; and %GC, from 35 – 39.3. Higher associated VLs tended to occupy the lower-left sections of the CpG/GC plane (Figure 1). Suggesting a negative effect of both traits on disease progression. R-squared values were: 0.01 for VL and 0.03 for CD4. Significant slopes confirmed visualizations, being negative for log₁₀(VL): -0.06 for GC (p<0.01), and -0.34 for CpG (p<0.001); and, positive for %CD4: 1.75 for GC (p<0.001) and 9.48 for CpG (p<0.001).

Conclusion: Linear models returned significant slopes but low R2, suggesting that CpG and GC architectures may significantly explain some of the VL and %CD4 variance; and that AS occupancy could return information on viral fitness not previously identifiable from VL or CD4. Architectural space can expand dimensions, and occupancy of pathogens could become a clinically relevant measure to assess potential virulence and transmissibility.
IL-12p70, TNF-α, IP-10, IFN-γ, TNF-β) cases had significantly lower values than controls at ENR but were nearly indistinguishable at X-1 (Figure). LASSO selected increase in IP-10, IL-7, IL-12p70, and IL-10 as jointly predictive of HIV acquisition, and relationships of these markers to outcome were modified by younger age, alcohol consumption, sexual role (versatile, receptive), having post-secondary education, and participating in sex work. Sensitivity analyses for time to HIV acquisition and potential for undetected eclipse-phase (HIV RNA-ve) infections at X-1 sampling did not change these results.

**Conclusion:** Unexpectedly, markers of immune activation in persons who acquired HIV in the subsequent month were indistinguishable from controls. However, almost all measured markers were significantly lower at enrollment in those who later acquired HIV; among cases, pre-infection levels in those with the largest increases did not exceed levels in controls at X-1. While changes over time may reflect regression to the mean, this study is novel in revealing that absolute changes in several markers were most predictive of HIV acquisition. This work may support a hypothesis that the process of activation, rather than chronic activation, increases risk. Further analysis of VirScan results in this study is ongoing and may shed light on viral infections that could explain these observations.

**Differences in markers of immune activation between persons who did or did not acquire HIV**

**A.** IL-12p70 levels at ENR

mean -0.51 log pg/ml in cases vs -0.028 log pg/ml, controls; p = 2.2 * 10^-12

**B.** IL-12p70 levels at X-1

mean 0.17 log pg/ml in cases vs 0.18 log pg/ml, controls; p = 0.92

**C.** tSNE with 10 cytokines at ENR

**D.** tSNE with 10 cytokines at X-1

**Figure:** Analysis of 10 cytokines measured by MSD in plasma showed drastic differences in most markers at ENR (A: IL-12p70 as an example) but not between last HIV negative visit or control (B). T-distributed stochastic neighbor embedding (tSNE) plots show overall clustering that has visually distinguished those who did or did not later acquire HIV (at ENR in C) but indistinguishable distributions on average 1 month prior to HIV acquisition vs control visit (D).

**253 SINGLE-CELL TRANSCRIPTOME ANALYSIS DURING PRIMARY HIV-1 INFECTION AND cART**

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**Background:** Treatment of HIV-1-positive individuals early in the course of infection provides the best opportunity for preservation of host immune function and limits the size of the latent HIV-1 reservoir. However, the transcriptional dynamics of discrete cellular subsets during treatment for primary HIV-1 infection has not been described in detail. We used single-cell RNA-sequencing to examine cell-type specific changes in gene expression in response to the initiation of cART during primary HIV-1 infection.

**Methods:** Using Drop-seq single-cell profiling, we generated longitudinal, unbiased gene-transcriptional profiles from cryopreserved PBMC of 10 HIV-1 positive MSM presenting during primary HIV-1 infection (TP1) and after approximately 1 year of uninterrupted, suppressive cART with PI or NNRTI based regimens (TP2). Mean age was 36 (24-44) years at initial presentation. Median estimated duration of HIV-1 infection at TP1 was 60 days (30-130). At TP1, individuals had lower median CD4/CD8 T cell ratios (0.47 versus 1.22) and higher HIV-1 RNA levels (Log 10 5.6 vs. < 1.7) compared to TP2, at which time all individuals were virologically suppressed (p<0.05 for all comparisons). GSEA identified gene pathways significantly modulated by cART.

**Results:** After strict quality control and filtering, our analysis identified 27,388 cells. The mean read depth per cell was approximately 43,000–84,000 as reported by CellRanger. Clustering using Seurat identified 10 clusters using expression of classical cell type markers (Fig 1). We then compared abundance of cell populations and transcriptional profiles within specific cell populations before and after cART initiation. As expected, we identified increases in the CD4/CD8 ratio after cART in all participants. In the CD4+ T cell, cytotoxic T-cell and B-cell compartments, gene pathways significantly down-regulated after cART initiation include inflammatory, complement, interferon-α and interferon-γ and the G2/M checkpoint (adj p < 0.05). In NK cells, novel significant down-regulation of EZH targets essential for DNA replication and cell cycle progression was also identified.

**Conclusion:** Single-cell transcriptional profiling of cohorts of HIV-1 positive individuals initiating cART during primary infection can provide an immunologic picture of an idealized immune response to cART and identify cell-type specific and global modifiable pathways of immune reconstitution.

**254 EARLY ART INITIATION MAY PRESERVE INFLUENZA VACCINE RESPONSE DURABILITY**

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**Background:** Aging and immunosenescence predict poor influenza vaccine responsiveness. We sought to determine whether treated HIV is also associated with poor vaccine responsiveness, and if delayed antiretroviral therapy (ART) initiation or persistent immune activation affect vaccine response in this setting.

**Methods:** People with HIV (PWH) with ART-mediated viral suppression > 1 year and HIV- controls, all CMV+ and enriched for HIV risk factors, received the seasonal flu vaccine 2014-2018. Total IgG titer against each year’s vaccine antigens were assessed at baseline, 1, and 4 months (M1 and M4, respectively). PWH were stratified by timing of ART initiation (within 6 months of HIV infection [early ART] vs later), and among later initiators, by nadir CD4 count (>350, 200-350, <200 cells/ml). Plasma KT ratio, IP-10, sCD14, sCD163, IL-6, STNF2, and sUPR were assessed at baseline. Antibody titer changes after vaccination were assessed with linear mixed-models, adjusted
for demographics, health-related behaviors, and timepoint-by-group and biomarker-by-timepoint interaction terms.

Results: Of 164 PWH and 41 HIV- participants, median age was 54 years and 91% were men. Of the HIV-, 56% were MSM, 34% were current smokers, 15% had distant IDU, and 41% had >100 lifetime sexual partners. Of the PWH, 34 were early ART initiators, and the remainder had a range of nadir CD4 counts: >350 (n=32), 200-350 (n=43), and <200 cells/ml (n=55). Median duration of viral suppression was 6 years. Flu-specific IgG titers increased from baseline to M1 similarly in all groups. While there was no evidence for titer decay M1 to M4 in HIV- and early ART initiators, later ART initiators experienced significant declines (P<0.04 for all). The extent of titer decay M1 to M4 was impacted by ART initiation timing: compared to HIV-, the early ART group had a similar slope of decay (P=0.66), but the combined later ART groups experienced a significantly greater rate of decline (P=0.02). IP-10 and sCD163, but not other biomarkers, were associated with a 9% greater rate of titer decline M1 to M4 per 1 IQR increase in either biomarker (P=0.05 and P=0.03).

Conclusion: ART-suppressed PWH have similar early humoral responses to influenza vaccination compared to HIV- adults, but only those who start ART within 6 months of infection appear to maintain similar response durability at 4 months. While the clinical implications of these findings remain unclear, some immune activation pathways appear associated with shorter response durability.

Methods: HIV infected ART-naïve subjects (N=8, 7 male) underwent ART in natural history trials at NIH. HIV cell associated (ca) DNA from peripheral blood lymphocytes (PBLs) prior to and after 6 days of ART was quantified using ddPCR assays targeting the LTR, and the numbers of cells infected daily were estimated from the numbers of infected cells lost during the 6-day interval. Plasma HIV RNA was measured (bDNA) frequently during this period. HIV caRNA was quantified for a subset (N=3) using qPCR of HIV gag in single infected cells obtained by end point dilution. Standard mathematical models were used to determine half-lives, HIV RNA production per cell, and reproductive ratio (R0).

Results: Only a small fraction of all HIV DNA positive cells detected in PBLs were lost per day after ART initiation, from which we estimated an average of 8.5 ± 107 cells infected/day/individual (range 5.0-13 x10^7). From the >40-fold decay of viremia during the first week of therapy, we estimated median daily virus production of 5.1 x 10^5/10^6 cells/virus/cell and R0 of 47 (range 12-92). Single infected cell analysis revealed that, prior to ART, 71-84% of all DNA positive cells produced HIV RNA; the majority containing c. 3 copies; a minority (1-1.8%) were "high-expressers" with 25-303 copies HIV RNA/cell. After 6 days of ART, only 17-21% of HIV DNA-containing cells contained HIV RNA, and levels of high expressors declined markedly. The half-life of high expressing cells (1.8 d) was similar to that of HIV producing cells measured independently by analyzing decay of plasma viremia (1.2 d).

Conclusion: The numbers of cells infected daily is a small fraction of all HIV DNA positive cells. A small fraction of all RNA positive cells contain high HIV RNA copy numbers and have a short half-life suggesting that these high expressing cells in peripheral blood are responsible for producing plasma viremia.
257 SARS-CoV-2 NEUTRALIZING-ANTIBODY RESPONSES IN CONVALESCENT INDIVIDUALS IN US AND PERU
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Background: SARS-CoV-2 has claimed over a million lives and remains a global threat. Understanding immune responses to infection and developing validated laboratory assays to measure them is critical to the rapid development, assessment and implementation of effective interventions. Our development of a validated pseudovirus neutralization assay and characterization of neutralizing antibody (nAb) profiles in a diverse post-SARS-CoV-2 cohort can inform preventative and therapeutic efforts, including vaccine and monoclonal antibody development and deployment.

Methods: This analysis comprises an observational cohort of n=330 adults in the US (n=168) and Peru (n=162), convalescing from SARS-CoV-2 infection and underlying co-morbidities, and peak approximately one month post-diagnosis. Large, diverse, well-characterized cohorts of convalescent individuals facilitate development of standardized laboratory methods and reagents to measure immune responses and provide standardized values to benchmark SARS-CoV-2 vaccine-elicited responses.

Results: The mean age is 48 years; 49% were assigned female sex at birth, 51% male; 54% are Latinx; 50% identified as Other race, 34% White, 11% Black, 4% Asian. The mean days from SARS-CoV-2 diagnosis to enrollment was 52. Nab titers were higher in participants with a history of severe illness (p<0.001) and peaked 28-42 days post-diagnosis. ID50 (ID80) nAb titers >20 were detected at enrollment in 66% (46%) of asymptomatic, 86% (74%) of symptomatic and 95% (92%) of hospitalized individuals. Median ID50 (ID80) titers at enrollment among asymptomatic, symptomatic and hospitalized individuals were 107 (10), 482 (59) and 1,953 (366), respectively. Two months post-enrollment, median ID50 (ID80) titers among asymptomatic, symptomatic and hospitalized individuals declined to 30 (10), 130 (16) and 564 (103), respectively. Diabetes (p=0.011), age >55yo (p<0.001), male sex (p=0.003) and BMI ≥30 (p=0.021) were associated with higher ID80 titers. Hypertension was associated with lower ID50 titers (p=0.005).

Conclusion: Nab titers after SARS-CoV-2 infection correlate with illness severity and underlying co-morbidities, and peak approximately one month post-diagnosis. Large, diverse, well-characterized cohorts of convalescent individuals facilitate development of standardized laboratory methods and reagents to measure immune responses and provide standardized values to benchmark SARS-CoV-2 vaccine-elicited responses.

258 PERFORMANCE AND DYNAMIC CHANGE IN SARS-CoV-2 ANTIBODY RESPONSES
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Background: Although reports suggest that most individuals with COVID-19 infection develop detectable antibodies post infection, the kinetics, durability, and relative differences between IgM and IgG responses remain poorly understood beyond the first few weeks after symptom onset.

Methods: Within a large, well-phenotyped, diverse, prospective cohort of subjects with and without SARS-CoV-2 PCR-confirmed infection and historical controls derived from cohorts with high prevalence of viral coinfections and samples taken during prior flu seasons, we measured SARS-CoV-2 serological responses (both IgG and IgM) using three commercially available assays. We calculated sensitivity and specificity, relationship with disease severity and mapped the kinetics of antibody seropositivity and antibody levels over time using generalised additive models.

Results: We analysed 1,001 samples (327 confirmed SARS-CoV-2, of whom 30% developed severe disease) from 752 subjects spanning a period of 90 days from symptom onset. Overall sensitivity was lower (44.1-47.1%) early (<10 days) after symptom onset but increased to >80% after 10 days. IgM positivity increased earlier than IgG-targeted assay but positivity peaked between day 32 and 38 post onset of symptoms and declined thereafter, a dynamic that was confirmed when antibody levels were analysed and was more rapid with IgM. Early (<10 days) IgM but not IgG levels were significantly higher in those who subsequently
developed severe disease (signal / cut-off 4.20 (0.75-17.93) versus 1.07 (0.21-5.46), P=0.048).

**Conclusion:** This study suggests that post-infectious antibody responses in those with confirmed COVID-19 infection begin to decline relatively early post infection and suggests a potential role for higher IgM levels early in infection predicting subsequent disease severity.

**Results:** Most study participants developed a neutralizing humoral response against SARS-CoV-2, however the maximum neutralization titer was 10-fold lower in mild/asymptomatic individuals compared to those with a more severe illness. We observed a slow and progressive decay of neutralizing activity in individuals with mild or asymptomatic disease throughout the 6-month period. In hospitalized individuals, half maximal neutralization activity was achieved on day 10 and showed an initial rapid decline that significantly slowed and remained nearly flat after day 80. Despite this, activity at six months remained higher in hospitalized individuals compared to mild symptomatic participants. On the other hand, we observed that IgG antibody titers against S2, RBD and NP had a more marked fall without showing differences in the decay pattern between individuals with different degree of severity of the disease.

**Conclusion:** Our data suggest that the neutralizing activity remains relatively stable for more than 6 months despite the decline in IgG antibodies, suggesting that the quality of immune response evolves and allows maintaining the neutralizing activity despite the decay in antibody titers. Our results provide a more detailed picture of the behavior of the natural humoral immune response over time that complements the current evidence on mid-term immunity.

**Background:** Immune dysfunction characterized by lower antibody (Ab) response to infection or vaccination has been well described among People Living with HIV (PLWH), but due to the novelty of the SARS-CoV-2 virus has not been evaluated among PLWH coinfected with SARS-CoV-2. This study compared the magnitude and longevity of Ab response to SARS-CoV-2 in a group of HIV+ and HIV- individuals infected with SARS-CoV-2

**Methods:** 17 HIV+ COVID-19+ and 19 HIV-COVID-19+ participants were recruited from the community as part of the ACTION study and followed longitudinally at day 14, 1 month and 3 months. HIV+ were on effective ART (plasma viral load <500 copies/ml), SARS-CoV-2 infection was confirmed by SARS-CoV-2 DNA PCR and rapid antibody test. All participants had mild/moderate COVID-19 without hospitalization. Antibody responses (IgG and IgM) were measured using an indirect in house developed ELISA using spike RBD antigen (courtesy, Scott Boyd, Stanford University) and the data are expressed as relative Ab units based on the positive control standard.

**Results:** The median age of HIV+ participants was 55 (26-63) with 23.5% (4/17) females. The median age for HIV- was 38 (27-78) with 57.8% (11/19) females. Time from COVID-19 diagnosis was 26 days for HIV+ and 21 for HIV-. Mean CD4 count for the HIV+ participants was 859.5 ± 287.2 cells/µL. Longitudinal analysis did not show a significant reduction in Ab response at 3 months in either HIV+ or HIV- groups. Levels of SARS-CoV-2 RBD specific IgM and IgG responses did not differ significantly between HIV+ and HIV- at any timepoint although there was a trend of lower IgM and IgG responses at 3 months in both groups compared to entry levels. Age was correlated with IgG response at day 14 (r =0.6, p = 0.02), 1 month (r =0.6, p = 0.014) and 3 month(r =0.7, p = 0.0008) in HIV+ and weakly correlated at day 14 (r =0.46, p = 0.04) in HIV-. Absolute CD4 count was not correlated with IgM and IgG responses in HIV+.

**Conclusion:** The magnitude and persistence of Ab response to SARS-CoV-2 infection in the 3–4 months post-infection does not differ by HIV status. Although extended longitudinal follow-ups are required to gain insights about the longevity of Ab responses in HIV+ individuals, results suggest that immune protection and vaccine responses may not differ by HIV status.
261 BINDING SIGNATURES AND CROSS-REACTIVITY IN THE SARS-CoV-2 IMMUNE RESPONSE
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Background: Mounting evidence indicates that antibodies generated during SARS-CoV-2 infection are correlates of protection. Antibodies targeting Spike (S) on the viral surface have been shown to neutralize the virus. However, the full repertoire of neutralizing and non-neutralizing antibodies against SARS-CoV-2, as well as cross-reactivity between SARS-CoV-2 and other circulating (CoVs), remains unclear. We sought to profile the complete repertoire of linear CoV epitopes targeted by the humoral immune response in patients with and without COVID-19 from Seattle, WA.

Methods: To map the linear epitope profiles in patients, we developed a comprehensive pan-CoV phage display library composed of 39 amino acid peptides covering the complete genomes of SARS-CoV-2 and the six other CoVs known to infect humans. Using samples from patients with confirmed COVID-19 and with no known SARS-CoV-2 exposure, we immunoprecipitated antibodies against CoV peptides, deep sequenced the co-immunoprecipitated phage, and applied a customized computational pipeline to define SARS-CoV-2 and cross-reactive epitopes.

Results: The dominant immune responses to SARS-CoV-2 were targeted to regions spanning N, Nucleocapsid (N), and ORF1ab. We identified 17 epitopes within S that were present in two or more individuals, spanning both the S1 and S2 subunits, with some detected in >75% of individuals. The most commonly mapped S epitope (S residues 1121-1159) was a region just upstream of the second heptad repeat. We identified nine epitopes within N that were reactive in at least two individuals, four of which were present in at least 35% of patients. The two most prominent N epitopes were derived from the RNA binding domain (N residues 141-179 and 161-199). Epitopes isolated from ORF1ab were the most variable across patients. Of the 46 unique ORF1ab epitopes we identified, only five were present in two or more individuals, suggesting that ORF1ab responses are individual-specific. We also found a high degree of variation in the total number of epitopes targeted by individuals (ranging from 2 to 25). Finally, we identified four unique cross-reactive sequences that were bound by antibodies in SARS-CoV-2 unexposed individuals.

Conclusion: Our study comprehensively defined the linear epitope profiles of a population of COVID-19 and SARS-CoV-2 unexposed patients. Epitope maps and functional characterization of SARS-CoV-2 antibodies will be critical for the development of a broad repertoire of COVID-19 treatments and vaccine strategies.

262 CHARACTERIZATION OF SARS-CoV-2–SPECIFIC RESPONSES IN PEOPLE LIVING WITH HIV
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Background: There is an urgent need to understand the nature of immune responses mounted against SARS-CoV-2, in order to better inform risk-mitigation and vaccine strategies for people living with HIV (PLWH).

Methods: To map the linear epitope profiles in patients, we developed a comprehensive pan-CoV phage display library composed of 39 amino acid peptides covering the complete genomes of SARS-CoV-2 and the six other CoVs known to infect humans. Using samples from patients with confirmed COVID-19 and with no known SARS-CoV-2 exposure, we immunoprecipitated antibodies against CoV peptides, deep sequenced the co-immunoprecipitated phage, and applied a customized computational pipeline to define SARS-CoV-2 and cross-reactive epitopes.

Results: The dominant immune responses to SARS-CoV-2 were targeted to regions spanning N, Nucleocapsid (N), and ORF1ab. We identified 17 epitopes within S that were present in two or more individuals, spanning both the S1 and S2 subunits, with some detected in >75% of individuals. The most commonly mapped S epitope (S residues 1121-1159) was a region just upstream of the second heptad repeat. We identified nine epitopes within N that were reactive in at least two individuals, four of which were present in at least 35% of patients. The two most prominent N epitopes were derived from the RNA binding domain (N residues 141-179 and 161-199). Epitopes isolated from ORF1ab were the most variable across patients. Of the 46 unique ORF1ab epitopes we identified, only five were present in two or more individuals, suggesting that ORF1ab responses are individual-specific. We also found a high degree of variation in the total number of epitopes targeted by individuals (ranging from 2 to 25). Finally, we identified four unique cross-reactive sequences that were bound by antibodies in SARS-CoV-2 unexposed individuals.

Conclusion: Our study comprehensively defined the linear epitope profiles of a population of COVID-19 and SARS-CoV-2 unexposed patients. Epitope maps and functional characterization of SARS-CoV-2 antibodies will be critical for the development of a broad repertoire of COVID-19 treatments and vaccine strategies.

263 ESCAPE OF SARS-CoV-2 501Y.V2 VARIANTS FROM NEUTRALIZATION BY CONVALESCENT PLASMA
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Background: New SARS-CoV-2 variants with mutations in the spike glycoprotein have arisen independently at multiple locations and may have functional significance. The combination of mutations in the 501Y.V2 variant first detected in South Africa include the N501Y, K417N, and E484K mutations in the receptor binding domain (RBD) as well as mutations in the N-terminal domain (NTD). Here we address whether the 501Y.V2 variant could escape the neutralizing antibody response elicited by natural infection with earlier variants.

Methods: We were the first to outgrow two variants of 501Y.V2 from South Africa, designated 501Y.V2.HV001 and 501Y.V2.HVdF002. We examined the neutralizing effect of convalescent plasma collected from adults hospitalized with COVID-19 using a microneutralization assay with live (authentic) virus. Whole genome sequencing of the infecting virus of the plasma donors confirmed the absence of the spike mutations which characterize 501Y.V2. This observation indicates that 501Y.V2 may escape the neutralizing antibody response elicited by natural infection with earlier variants.

Conclusion: This observation indicates that 501Y.V2 may escape the neutralizing antibody response elicited by natural infection with earlier variants.
overcome these current limitations, we performed a high-resolution mapping using IFN-γ ELISpot and peptide sets covering the entire CoV-2 proteome.

**Methods:** We synthesized a 15-mer peptide library of 2790 peptides (11 amino acid overlapping) covering a CoV-2 conserved proteome sequence based on 1700 sequences. We designed a mega matrix of consecutive and non-consecutive peptide pools with 20 to 35 peptides per pool. We assessed T-cell responses in cryopreserved PBMCs from IgG+ SARS-CoV-2 infected individuals (N=13), who recovered from mild/moderate infection, 90-190 Days from off-set symptoms. Also, we expanded PBMCs in the presence of anti-CD3 and IL-2 during 3 weeks and performed a comparative ELISpot using total and expanded PBMCs.

**Results:** Frequencies of T-cell responses from positive peptide pools revealed 40% of responses targeting S2, 20% against S1, 10% against M, and 6% against nsp3 and NP, respectively. The strongest responses were targeting S2 and S1 (median values of S40 and 240 IFN-γ SC/10^5, respectively), followed by nsp3, NP and M. We observed a median of 13 deconvoluted reactive peptides across the entire proteome per tested individual. The breadth of responses ranged from 1-8 targeted proteins with a median of 2. In addition, we mapped responses in subproteins 3C-LP, nsp6, nsp10 (OrfTab), and alternative reading frames. We also identified responses to peptide sequences conserved across pan-coronavirus strains Orf1b (n=2), S (n=1) and M (n=1). Following expansion, we observed a loss of CD4+ T-cells in cultured cells and altered peptide-recognition profiles characterized by a loss of S2 and an increase of nsp3 responses.

**Conclusion:** We characterize protein hierarchy in terms of breadth and magnitude by high-resolution mapping of T-cell responses against the entire CoV-2 proteome. The most frequently targeted and immunogenic regions were S2 and S1. We identify responses to small proteins, alternative reading frames and conserved regions across coronaviruses. This data brings new insight into the complexity of CoV-2 T-cell responses and crucial information for vaccine design.

265 SARS-CoV-2 NON-SEROCONVERTORS PRESENT T-CELL RESPONSES WITH DECREASED ACTIVATION

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**Background:** Many immune studies of SARS-CoV-2 (CoV-2) infection have focused on the generation of virus-specific as a means of protection. However, a small group of CoV-2 infected individuals called Non-seroconverters (NSC), do not generate antibodies but experience a mild or moderate disease course. Identifying mechanism of CoV-2 control in NSC may inform the development of novel therapeutics and vaccines approaches.

**Methods:** We identified eleven CoV-2 NSC (3.6%) from the King-cohort study (PI-20-217). NSC were defined by a positive CoV-2 PCR at the time of diagnosis in the absence of IgG, IgA and IgM in serum and plasma measured by two independent ELISA techniques. For comparison, we identified groups of CoV-2 convalescent (n=15) and low-neutralizers (n=15). We measured T-cell responses to the CoV-2 Spike (S) and Nucleocapsid (NP) recombinant proteins in PBMCs by ELISPOT and flow cytometry. We combined T-cell surface and lineage markers together with PD-1, functional (TNF, IFN-γ, and IL-2) and activation markers (AIM: CD25, CD137 and OX40).

**Results:** We identified CoV-2 specific CD4+ and CD8+ T-cells against the S and NP in NSC individuals. All NSC responded to S by production of one or more cytokine in either CD4+ or CD8+ T-cells, and 57% responded to NP. Specific CD8+ T-cells against S in NSC were characterized by IFN-γ and TNF production, and we observed higher levels of TNF production as compared to low neutralizers (p=0.02). No differences were found in IFN-γ, IL-2 and TNF production in S-specific CD4+ T-cells between groups, nor in NP CD8+ or CD4+ T-cell responses. The levels of CD137/CD4+ in CD8+ and CD4+ T-cells were significantly lower in NSC in response to S (p=0.006, and p=0.012). Also, lower levels of PD-1 were observed in CD8+ T-cells in response to NP in NSC (p=0.017).

**Conclusion:** We provide evidence of SARS-CoV2 cellular immunity in NSC individuals despite the absence of humoral neutralizing responses. CD8+ and CD4+ T cells against the S and NP were present in NSC and characterized by TNF production in CD8+ T-cells in response to S when compared to low neutralizers. Decreased levels of activation markers were observed in NSC’s following S and NP stimulation. We propose a protective role of cellular immunity in NSC potentially driven by preexisting cellular responses.
Ebola, GP (Grp1). Only four out of ten animals in this vaccinated Grp1 group were infected by the 7th challenge. Eight of 10 in the control macaques (Grp3) and 10 of 10 alternative vaccine group (Grp3) were infected with SHIV SF162-p3 during the 7 low dose challenges.

**Conclusion:** Building on the VSV-Ebola vaccine technology, we engineered an improved VSV-vectored vaccine for HIV prevention. We observed one of the best protections against low dose heterosexual SHIV challenges in macaques to date. Analysis on macaque samples suggest that the use of VSV-based Env construct with Gal may be important for protection, especially in boosting CTL response, but its inclusion in the final boost (groups 2 and 3) may promote an activated CD4+ T cell population capable of increasing SHIV infection. These findings suggest an important balance of cell-based versus humoral immunity in the use of vaccine constructs in prime/boost strategies for optimal protection.

**268 DESIGN AND IMMUNOGENICITY OF V3-GLYCAN EPITOPE-FOCUSED NANOPARTICLES FOR HIV VACCINES**

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**Background:** Induction of broadly neutralizing antibodies (bnAbs) that confer protection against diverse strains is a primary goal of HIV-1 vaccine design. In order to accomplish this feat, antibodies must target conserved sites on the Env (Env) surface. The V3-glycan epitope is a well-defined site of vulnerability targeted by a class of antibodies with extreme neutralization potency and breadth. We designed minimal nanoparticle-based immunogens that recapitulate this conserved site to focus the immune response on the V3-glycan epitope. We hypothesize vaccination with minimal immunogens will target and expand subdominant neutralizing antibodies (nAbs) without boosting dominant strain-specific non-nAbs.

**Methods:** V3-glycan epitope nanoparticles were produced in Freestyle293 cells and purified by 2D12 affinity chromatography. Formation of nanoparticles was determined by negative stain electron microscopy. Immuno-gen recognition by bnAbs was assessed by ELISA. Immunogenicity studies were performed using female New Zealand white rabbits. Rabbits (n=5 per group) were immunized intramuscularly with either SOSIP or adjuvant at week 0, 4 and 8. Animals received boosts with the V3-glycan epitope nanoparticle at weeks 12 and 22. Serum was collected 2 weeks after each immunization. Serum antibody binding titers were determined by ELISA. Epitope mapping of serum antibodies was assessed by decreases in binding to Env with mutated epitopes. Serum nAbs were determined by the TZM-bl assay using pseudoviruses.

**Results:** We generated ferritin nanoparticles that each display 24 copies of a glycopeptide that mimics the V3-glycan epitope. These nanoparticles are antigenic for V3-glycan bnAbs but not linear V3 antibodies. Binding of the bnAbs was further enriched by enriching for MamuG6/NA2 protein glycosylation. Rabbits received three immunizations with a group M consensus stabilized Env SOSIP followed by two boosts with the glycan-V3 ferritin nanoparticles. A single boost elicited antibodies with preferential binding to the glycopeptide versus the aglycone peptide. A second boost increased glycopeptide binding, but also induced V3 peptide-specific antibodies. Boosting sustained titers of SOSIP-specific antibodies for 22 weeks–12 weeks after the final trimer immunization.

**Conclusion:** Glycer-V3 nanoparticle vaccination elicited V3-directed antibodies that were predominantly glycan-dependent. Our results establish a nanoparticle platform by which minimal immunogens can be used to target antibodies to a specific epitope.

**269 NANOPARTICLE-DCs RESTORE CYTOTOXIC MEMORY-LIKE NK CELLS IN CHRONIC HIV PATIENTS**

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**Background:** Cytotoxic CD16+ Natural Killer (NK) cells become dysfunctional in HIV-1 chronically infected individuals even after antiretroviral therapy initiation, preventing effective elimination of HIV-1 infected cells. Previous studies suggest that activated dendritic cells (DC) stimulate NK and might be useful for therapeutic purposes. However, DCs from chronic HIV-1 patients can also be unresponsive to adjuvant stimulation. Thus, new approaches are needed to maximize DC-NK crosstalk in chronic HIV-1 patients. Here, we evaluated the efficacy of DC conditioned with nanoparticles containing Poly I:C preserving cytotoxic function of CD16+ NK cells from HIV-chronic patients.

**Methods:** Monocyte-Derived Dendritic Cells (MDDCs) were derived from peripheral blood monocytes from aviremic treated chronic HIV-1 patients or from healthy donors in the presence of GMCSF and IL4 for 5 days. MDDCs were exposed to either soluble or nanoparticle-loaded Poly I:C (PIC) and expression of inflammatory cytokines and NK cell receptor ligands was determined after 24h by multicolor flow cytometry. Unstimulated or nanoparticle-treated MDDCs were co-cultured with autologous NK cells and expression of CD107a, NKGC2 and CD57 was also determined by FACS. Natural cytotoxic function of NK cells was assessed by coculture with the target cell line K562-GFP. Finally, NK cells from treated HIV patients were incubated with autologous CD4+ T cells in the presence of IL-2, Raltegravir and Romidepsin. Proportions of HIV-I p24+ CD4+ T cells detected after treatment with NK cells was evaluated after 24h by FACS.

**Results:** MDDC treated with PIC nanoparticles express higher levels of IL-12 and IFN-β (p=0.03; p=0.03) and MICA, ULBP1 ligands for NK receptors, in contrast to MDDC treated with soluble PIC. MDDCs conditioned with PIC-nanoparticles were capable of inducing higher frequencies of cytotoxic CD107a+ CD16+ NK cells (p=0.01) characterized by effective natural cytotoxic function (p=0.01). Importantly, MDCRs from chronic HIV-1 patients also express higher levels of activating NK receptor ligands (p=0.0001), increased proportions of memory-like NKG2C+ CD57+ CD16+ NK cells (p=0.03) and induced a more effective reduction of autologous HIV p24+ expressing CD4+ T cells (p=0.01) after PIC-nanoparticle treatment.

**Conclusion:** Conditioning of DC with PIC nanoparticles is a promising tool to restore natural cytotoxic function of CD16+ NK cells from chronic ART-treated HIV-1 patients and more efficiently targeting HIV-1 infected CD4+ T cells.

**AAV-EXPRESSED ANTI-HIV BIOLOGICS BLOCK ORAL SHIV ACQUISITION IN INFANT RHESUS MONKEYS**

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**Background:** Antiretroviral therapy has been highly effective in limiting mother-to-child transmission of HIV but has suffered from problems related to accessibility and compliance. Given these issues, we have begun studies in infant rhesus macaques (RMs) to assess the potential of long-term delivery of anti-HIV biologics using adenovirus-associated virus (AAV) as a vector for the prevention of postpartum HIV transmission.

**Methods:** Nine newborn RMs were evenly divided among 3 groups depending on which AAV treatment they received. Group 1 was treated intramuscularly (IM) with an AAV vector encoding the immunoadhesin eCD4-Ig. Group 2 was dosed with AAV8/eCD4-Ig intravenously at birth, followed by an IM dose of AAV1/eCD4-Ig four weeks later. Group 3 was inoculated IM with an AAV8 vector encoding the broadly neutralizing antibody 3BNC117. Beginning at week 30, the infants in Groups 1-3, together with 6 age-matched control RMs, were subjected to weekly oral challenges with escalating doses of SHIV-AD8. Serum levels of eCD4-Ig, 3BNC117, and anti-drug antibodies (ADAs) were measured by ELISA. Plasma viral loads were measured by real-time PCR.

**Results:** All AAV inoculations were well tolerated. All RMs in Groups 1 and 2 developed persistent serum levels of eCD4-Ig in the 12-70 µg/ml range. Although one animal in each group mounted ADAs, these responses did not abrogate eCD4-Ig expression. Of the three RMs in Group 3, two developed persistent levels of 3BNC117 in the 48-79 µg/ml range. The third animal mounted a robust ADA response that drove its 3BNC117 levels to below persistent levels of 3BNC117 in the 48-79 µg/ml range. The third animal mounted a robust ADA response that drove its 3BNC117 levels to below persistent levels of 3BNC117 in the 48-79 µg/ml range.

**Conclusion:** Neonatal delivery of AAV vectors encoding anti-HIV biologics can block oral SHIV acquisition in infant RMs. Given the potential of this strategy to generate broad, potent, and durable anti-HIV immunity after a single dose, studies aimed at evaluating the safety and antiviral efficacy of AAV- vectored HIV immunotherapy in human infants seem warranted.
271 PROTECTION FROM SHIV INFECTION IN IMMUNE-COMPLEX VACCINATED RHESUS MACAQUES

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Background: Immune complex (IC) vaccines have been reported to be more efficient in presenting antigens to the immune system and to promote cross-presentation of exogenous antigens on class-I MHC. Here, we tested the immunogenicity and efficacy of a virus-like particle (VLP)-based IC vaccine in a non-human primate model.

Methods: VLPs were produced by transfecting an SIV backbone into an HEK293 clone stably expressing HIV-1 Env (WITO). A4B7+ VLPs were produced by co-transfection of a4 and b7 subunits. Five groups of rhesus macaques (N = 5) were vaccinated by 6 sequential s.c. inoculations of pre-formed ICs over a period of 4 months, with an additional group serving as naive controls. ICs were formed with either a bNAb (VR263), or a primatized anti-a4b7 antibody (ACT-1, wild-type or Fc-defective), with two control groups receiving a4b7-negative VLPs mixed with ACT-1 or an irrelevant IgG. After vaccination, all animals were challenged by 12 repeated low-dose intrarectal inoculations of a non-pathogenic tier-1b SHIV, BaL, followed 3 months later by rechallenge with a pathogenic tier-2 SHIV, AD8. Viral Load and antiviral T-cell responses were monitored. ELISA and TZM-bl assay were used to test Env-specific antibody production.

Results: All animals in the control group were rapidly infected by SHIV-BaL reaching high levels of viremia, while the bNAb-IC group was partially protected with two animals remaining uninfected and the other three showing marked reductions of peak viremia. Four additional animals in three other vaccine groups remained uninfected; however, viremia in those infected animals reached regularly high levels. SHIV-BaL replication was rapidly controlled in all infected animals. Following rechallenge with SHIV-BaL, all animals previously uninfected with BaL were rapidly infected with AD8 reaching high levels of viremia. In contrast, half of those previously infected with BaL were completely protected from AD8, while the remaining showed significantly reduced and delayed viremia. Protection from AD8 superinfection was associated with robust Gag- and Env-specific T-cell responses. Antibody-mediated CD8a depletion caused a rapid viral rebound in all animals.

Conclusion: An IC vaccine with bNAbs provided partial protection against a non-pathogenic SHIV (BaL) in macaques. However, previous exposure to BaL conferred a strong protection from a pathogenic tier-2 heterologous strain (AD8). Virus-specific T-cell responses were correlated with protection, with a key role played by CD8 T cells.

272 DUAL-ANTIGEN COVID-19 VACCINATION WITH ORAL BOOST PROTECTS NHP FROM VIRAL CHALLENGE

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Background: To address the need for an efficacious COVID-19 vaccine suitable for worldwide distribution, we have developed a dual-antigen vaccine incorporating genes for a modified SARS-CoV-2 spike protein (S-Fusion) and the viral nucleocapsid protein (N) with an Enhanced T-cell Stimulation Domain (N-ETSD) with the potential to increase MHC class-I/II responses. The adenovirus serotype 5 platform used, hAd5 [E1-, E2b-, E3-] can be delivered in an oral formulation that overcomes cold-chain limitations. The hAd5 S-Fusion + N-ETSD vaccine was evaluated in rhesus macaques to determine both humoral and cell-mediated responses to vaccination, and protection from subsequent SARS-CoV-2 challenge.

Methods: Non-human primates (NHP) received either a subcutaneous (SC) prime and two oral boosts at 2-week intervals, or a SC and one oral boost (each group n = 5). There was also a placebo group (n = 2). Humoral responses to spike (S) were determined by ELISA and T-cell responses to S and nucleocapsid (N) by ELISPOT. Neutralization capability of sera was assessed by a surrogate assay and by a microneutralization assay. After SARS-CoV-2 challenge of 106 plaque-forming units (PFU) of SARS-CoV-2 strain 10012, viral RNA by Quantitative RT-PCR (qRT-PCR) were determined in nasal swab and bronchoalveolar lavage (BAL) samples by RT-qPCR.

Results: In response to hAd5 S-Fusion + N-ETSD vaccination, NHP generated SARS-CoV-2-neutralizing anti-spike (S) antibodies and demonstrated T-cell activation by both S and nucleocapsid (N). Both the subcutaneous (SC) prime followed by two oral boosts or an SC and oral boost protected the upper and lower respiratory tracts of non-human primates from high titer SARS-CoV-2 challenge. Notably, inhibition of viral replication began within 24 hours of challenge in both lung and nasal passages, becoming undetectable within 7 days post-challenge. Rapidly enhanced neutralization capability of sera in the two weeks after challenge suggests the presence of memory B cells that were activated by infection.

Conclusion: The hAd5 S-Fusion + N-ETSD vaccine, when given as a subcutaneous prime with oral boosts, protects rhesus macaques from subsequent viral challenge. The decrease in subgenomic RNA seen at the first time point for sample collection post-challenge (24 hours) provides evidence that protection was almost immediate. The thermally-stable oral form of the vaccine has the potential to facilitate global distribution of vaccines, especially in developing nations.

273 DISTINCT TISSUE TOPOLOGY AND CELL PHENOTYPES PREDICT NEUTRALIZATION IN HIV INFECTION

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Background: The induction of broadly neutralizing antibodies (bNAbs) is a major goal of HIV vaccine efforts. In chronic infection however, only a small percentage of HIV–infected individuals (~2%) is able to mount this type of humoral response and the lymph node (LN)–specific parameters associated with such outcomes in the context of persistent viremia remain to be elucidated. To address this question, we performed a detailed analysis of the topology and immunophenotype of LN-resident CD4 T–cell and B–cell populations in viremic HIV+ volunteers with and without broadly neutralizing activity profiles.

Methods: LN cell suspensions and matched formalin–fixed, paraffin embedded (FFPE) tissue sections derived from chronically infected HIV+ individuals were analyzed using 30–colour multiparametric flow cytometry and quantitative multiplexed confocal imaging. Neutralization activity of matched serum samples was determined using a single–round infectivity assay. Breadth of neutralization was defined by calculating the percent of HIV-1 Env–pseudoviruses that achieved an ID50 > 20

Results: The analysis of LN architecture and germinal center (GC) topology revealed greater follicular GC disruption in HIV+ non–neutralizers compared to HIV+ volunteers with and without broadly neutralizing activity profiles. In addition, a lower frequency of unswitched CD27hi IgD+ memory B cells was found in neutralizers compared to non-neutralizers when B-cell populations were examined (p=0.030).

Conclusion: Neutralizing activity is associated with a greater degree of follicular GC preservation in the LN of chronically infected HIV individuals, higher levels of Tfh differentiation and lower frequencies of unswitched memory B cells. Thus, the implementation of strategies directed at preserving GC architecture during viremia may hold particular promise for the generation of broadly neutralizing antibodies in individuals affected by HIV.

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TISSUE LANDSCAPE OF HIV ANTIBODY NEUTRALIZATION SUSCEPTIBILITY
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Background: HIV-1 genetic diversity and the presence of archived provirus that harbors escape mutations to antibodies are major obstacles for the clinical use of broadly neutralizing antibodies (bNAbs) to treat HIV-1 and for the use of bNAbs as interventions to clear reservoirs. This study aimed to characterize the viral reservoir to its susceptibility to antibody neutralization across peripheral blood mononuclear cells and deep tissues.

Methods: We analyzed near full length HIV env sequencing data generated from antemortem blood and postmortem tissues from participants in the Last Gift autopsy cohort using a Bayesian machine-learning model. The model uses HIV-1 envelope protein sequences and approximates glycan occupancy information as variables to quantitatively predict the half maximal inhibitory concentrations (IC50) of bNAbs. Using linear mixed effect models, this allowed us to map the landscape of neutralization resistance across each person’s tissue reservoirs for 9 distinct bNAbs (targeting the CD4 binding site, V3-glycan, V2-apex and MPER) and grouped tissue sites (i.e. central nervous system [n=4 sites], genital tract [n=4], gut [n=8] and lymph nodes [n=9]) within and across participants.

Results: We analyzed a total of 655 Env sequences (mean 109 [95%CI:72.7-145.6]/participant) across 32 distinct blood and tissue sites (mean 20 [95%CI:14.4-26.6] sequences/site) from 6 participants. We observed expected heterogeneity of predicted neutralization susceptibilities across participants but also across bNAb classes and tissues within participants. Neutralization susceptibilities were within the ranges that have been described for each tested bNAb, but for some antibodies, targeting the V2-apex or the V3-glycan, the predicted neutralization pattern differed between tissue compartments (p<0.001). In 5 participants that had remained suppressed on ART until their death, the breadth of neutralization susceptibilities in the PBMC reservoir did not differ from what was found in tissues. This observation was consistent across antibody target classes (P>0.1).

Conclusion: In persons with HIV (PWH) suppressed by ART, the landscape of predicted viral resistance to bNAb neutralization in the PBMC reservoir seems to match what is observed in tissues. These data suggest that sampling the blood might be sufficiently representative of the diversity of the viral reservoir within an PWH to facilitate the selection of personalized bNAb combinations for therapeutic approaches.

ANTIBODY PROFILING IDENTIFIES ANTIBODY TARGETS ASSOCIATED WITH NATURAL HIV CONTROL
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Background: HIV viral suppression is associated with delayed disease progression and reduced transmission. HIV controllers suppress HIV viral load to low levels without antiretroviral treatment (ART). We used a massively-multiplexed antibody profiling system (VirScan) to compare the antibody profiles in HIV controllers, viremic non-controllers, and non-controllers who were suppressed on ART. The VirScan assay provides quantitative information on antibody binding to >3,300 peptides spanning the HIV genome.

Methods: Antibody reactivity was assessed in 13 elite controllers, 27 viremic controllers, 21 non-controllers who were virally suppressed on ART, and 12 viremic non-controllers (Discovery Cohort). Antibody reactivity to selected peptides was quantified in a second cohort that included 29 elite controllers and 37 non-controllers who were virally suppressed on ART (Validation Cohort). Antibody reactivity was also assessed in 298 samples from 53 non-controllers who had viral load data from longitudinal visits that was used to determine viral load set point.

Results: In the Discovery Cohort, we identified 62 peptides that were preferentially targeted in HIV controllers compared to non-controllers. In the Validation Cohort, combined antibody reactivity to these peptides was also higher in elite controllers compared to non-controllers who were virally suppressed on ART. The reactivity of antibodies to the 62 peptides was similar.
among HIV controllers who did or did not have the protective HLA-B*57 allele. All but one of the 62 peptides were grouped into seven clusters of homologous peptides. The clusters were located in gp120 (two clusters), gp41, p17, p24, vpu, and integrase. Higher antibody reactivity to a subset of the peptides in the p17 cluster was significantly associated with lower viral load set points in the group of longitudinally-followed non-controllers.

Conclusion: A comprehensive, unbiased assessment of antibody reactivity to HIV peptides spanning the viral genome identified clusters of homologous peptides that were preferentially targeted in HIV controllers and non-controllers who had lower viral load set points. Further research is needed to characterize antibodies that target these peptides and evaluate T cell targeting of these epitopes. This research will provide new insights into natural control of HIV infection and may inform research on immune-based interventions for HIV prevention and treatment.

276 AUTOLLOGOUS NEUTRALIZING ANTIBODIES INCREASE WITH EARLY ART AND SHAPE HIV REBOUND

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Background: Early initiation of antiretroviral therapy (ART) alters viral rebound kinetics after treatment interruption (TI) and may play a role in reducing the barrier to HIV remission. However, little is known about the underlying mechanisms and the selection of rebounding variants. Autologous neutralizing antibodies (aNAbs) represent a key adaptive immune response against a broad range of viruses and in this study, we evaluated whether aNAbs can develop in the setting of early-ART initiation and investigated their role in shaping post-TI HIV rebound variants.

Methods: We performed single-genome amplification of HIV-1 env from pre-ART and post-TI plasma samples of 5 participants from the ACTG 371 study of early-treated individuals. aNAbs were quantified using pseudoviruses from the most common plasma sequences and the serum dilution that inhibits 50% of viral infections (ID50) was determined.

Results: We tested the ability of pre-ART and post-TI plasma to neutralize virus pseudoviruses derived by obtaining a median of 52 single-genome sequences from pre-ART and post-TI time points. Pre-ART, the neutralization was median 33,111 copies/ml and only weak aNAbs were detected. aNAbs targeted early post-TI plasma against pre-ART virus. All participants were virologically suppressed (median 44 weeks) prior to TI. aNAbs were expected to significantly while on suppressive ART as early post-TI plasma (median 8 weeks post-TI) demonstrated significantly improved neutralizing activity compared to pre-ART plasma (pre-ART vs early post-ATI ID50 plasma neutralizing titers [1/x]: median 20 vs 432, P<0.0001). Post-TI aNAbs responses exerted selective pressure on the rebounding viruses as the HIV variants detected during TI were significantly more resistant to post-TI plasma neutralization compared to pre-ART virus (early post-TI plasma neutralization titers [1/x]: 432 vs 37, P=0.046). Compared to the post-ART time point, viral diversity was also restricted during the TI (average pairwise distance of pre-ART vs post-TI plasma viruses: 0.09% vs 0.16%).

Conclusion: Early initiation of suppressive ART allows for the strengthening and maturation of the anti-HIV autologous neutralizing antibody response. Rebounding HIV variants are more resistant to contemporary neutralization, suggesting that viral variants contributing to viral rebound do not arise purely from a stochastic process, but are shaped by host immune pressures, including aNAbs responses.

277 FORCED RESIDENCY OF T CELLS IN VIREMIC TISSUES DOES NOT INDOUCE CONTROL OF HIV VIREMIA

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Background: HIV and SIV infected CD4+ T cells localize primarily to mucosal and lymphoid tissues (LT), which are also a major site for maintenance and subsequent recrudescence of the long-term HIV reservoir. Cytotoxic CD8+ T cells are critical for control of HIV and SIV viremia but the most potent peripheral blood cytotoxic CD8+ T cells are rarely found in LT, indicating that they seldom have the opportunity to interact with infected CD4+ T cells. Here, we assessed the impact of LT CD8+ T cells on SIV immunopathogenesis by inhibiting cell egress from LT in viremic rhesus macaques (RM) using the lymphocyte migration inhibitor FT720. We hypothesized that the retention of recirculating CD8+ T cells in LT may enable local differentiation into cytotoxic effector cells with a subsequent impact on plasma viral load.

Methods: Four RM were infected intravenously with SIVmac239, and treated with FT720 daily from day 7 or 28 until day 90 post-infection. Separately, fourteen acutely infected RM were treated with antiretrovirals (since day 14 post infection) for 6 months followed by treatment interruption while seven of them were receiving FT720. Peripheral blood and lymph node (LN) samples were collected for flow cytometry analyses and viral load quantification.

Results: We observed near complete redistribution of circulating T cells into tissues within 28 days of FT720 treatment (436±346 vs 75±62 CD8+ T cells/µl; and 610±462 vs 4±3 CD4+ T cells/µl). Despite the FT720-induced retention of CD8+ T cells in LT, no beneficial effect was observed on peak viremia or set point during acute infection, nor time or degree of viral rebound after antiretroviral treatment interruption. FT720-enforced tissue retention promoted an increase in the frequency of SIV-specific CD8+ T cells in LN of FT720-treated RM after antiretroviral treatment interruption (median 0.035 vs control RM). However, the frequency of perforin+ granzyme B+ cells within LN SIV-specific CD8+ T cells remained low (1±1% vs 2±2%, pre vs post FT720). Furthermore, FT720 treatment did not increase the frequency of follicular homing CXCR5+ SIV-specific CD8 T cells.

Conclusion: These data indicate that simply increasing the number of CD8+ T cells in LT is insufficient to enable viral control in the SIV model. Moreover, enforced retention of CD8+ T cells in viremic tissues does not enable the acquisition of cytotoxic properties, suggesting that secondary signals not present in LT may be necessary to promote or maintain cytotoxic CD8+ T cell differentiation.

278 CD8+ RESIDENT MEMORY T CELLS CONTROL THE HIV RESERVOIR IN THE CERVICAL MUCOSA

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Background: The major hurdle to HIV-1 eradication is the establishment of viral reservoirs. CD8+ T cells constitutively express cytotoxic molecules and their profile indicates that antiviral defense and target cell destruction represent key functions of these cells. Here we aimed to address the functional capacity of HIV-specific and non-specific CD8+ T RM from the cervical mucosa in limiting HIV viral persistence.

Methods: CD8+ T RM cells from cervical tissues were phenotyped by FACS (n=9). In ART-suppressed HIV+ women (n=8), we determined total vDNA in blood and cervix and its correlation with the frequency of cervical CD8+ T RM, as well as Gag-specific CD8+ T RM in cervical biopsies (n=7). A functional assay was established to assess suppression of reactivated CD4+ T cells by cervical CD8+ T RM from an ART-suppressed HIV+ woman undergoing hysterectomy. To evaluate non-specific natural capacity of CD8+ T RM in limiting the reactivated viral reservoir, an ex vivo latency model using IL-7 was established using cervical explants from uninfected women. Natural cytotoxicity was measured by simultaneously determining p24 expression and cell-associated HIV-1 DNA in reactivated CD4+ T cells in the presence or not of autologous CD8+ T RM from uninfected tissue.

Results: Cervical CD69 + CD8+ T cell profile was compatible with >90% belonging to bona fide TRM. Further, CD8+ T RM in cervical tissue represented >90% of CD69+ CD8+ T cells, and cervical samples from ART-suppressed patients had higher frequencies of CD8+ T RM (p<0.05) and non-TRM (p<0.01). The frequency of cervical CD8+ T RM cells correlated with proviral HIV-1 DNA in cervix (n=7; p=0.03) and blood (p=0.05). Gag-specific CD8+ T RM were rarely detected in biopsies, which was likely limited by sample size. Still, cervical CD8+ T RM cells from the HIV-infected woman with a large sample were more
efficient at eliminating HIV-reactivated CD8+ T cells than circulating effector CD8+ T cells. The latency model evidenced a natural capacity of CD8+TRM to reduce p24+ cells after reactivation (n=7; p=0.08; Figure), which, in cases of higher cell yield recovery, was associated with a decrease of the total reservoir size.

Conclusion: CD8+TRM in cervix are associated with less proviral HIV-1 DNA and may exert a greater control of the reservoir than effector circulating CD8+ T cells. A cervical latency model could be crucial to study how HIV tissue reservoir could be eliminated not only by enhancing HIV-specific CD8+TRM but also by promoting their natural cytotoxicity.

279 EVALUATING VIRUS-SPECIFIC CD8 T CELLS FROM MULTIPLE ANATOMICAL SITES

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Background: Virus-specific CD8 T cells are critical for control of SIV and HIV viral replication. SIV infection in Rhesus macaques (Macaca mulatta) induces SIV-specific CD8 T cells expansion in the blood and multiple tissues. To further evaluate the mechanisms underlying the induction and maintenance of SIV-specific CD8 T cells, we determined the kinetics of SIV-specific CD8 T cells across multiple anatomical sites during acute infection, chronic infection and treatment with anti-retrovirals. Furthermore, we utilized an SIV-gag DNA vaccine to assess the induction of SIV-specific cells upon acute antigen exposure. Finally, we utilized cytomegalovirus (CMV) infected animals to compare the tissue distribution of virus-specific CD8 T cells between two viruses that induce a chronic infection in the host.

Methods: Mamu-A*01+ or Mamu-*02+ Rhesus macaques were infected with SIVmac239 or administered with 1 mg of DNA plasmid CMV/R-SIVgag vaccine. CMV infection occurred naturally in the animal facility. SIV-specific CD8 T cells were enumerated and sorted by FACS from the PBMCs, bronchoalveolar lavage (BAL) lymph nodes (LN), liver, spleen, colon and jejunum using MHCI Pentamers. We assessed the induction of SIV-specific cells upon acute antigen exposure. Finally, we utilized cytomegalovirus (CMV) infected animals to compare the tissue distribution of virus-specific CD8 T cells between two viruses that induce a chronic infection in the host.

Results: Acute SIV exposure induces virus-specific CD8 T cells in peripheral blood and lymph nodes, while chronic viremia seems to be required for expansion in other tissues. This expansion is maintained after administration of antiretroviral therapy, with a tissue resident phenotype most common across the gastrointestinal tract. Finally, natural CMV infection induced CMV specific CD8 T cells predominantly in the PBMCs compared with other anatomical sites.

Conclusion: SIV-specific CD8 T cells can inhabit multiple anatomical sites upon both acute and chronic antigen exposure, with a small fraction developing a tissue-resident phenotype. In comparison, CMV infection induces CMV-specific CD8 T cells primarily in the blood, suggesting distinct tissue distribution between different viruses that induce chronic infection. Clonotypic analysis reveals potential mechanisms involved in development of tissue-specific immunological phenomena during viral infections.

280 COMBINATION OF IMMUNE CHECKPOINT BLOCKADE INCREASES IL-2 IN HIV-SPECIFIC CELLS

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Background: In people with HIV (PWH), elevated expression of immune checkpoints (IC) persists despite ART, leading to T-cell exhaustion. We aimed to determine if blocking single or multiple IC would enhance HIV-specific T cell function ex vivo.

Methods: Bulk PBMC obtained from 11 PWH on suppressive ART were stimulated with either gag, nef or Cytomegalovirus, Epstein-Barr Virus and Influenza (CEF) peptides in the presence of blocking antibodies to six IC molecules, including CTLA-4, PD-1, PD-L1, TIM-3, TIGIT and LAG-3 or relevant isotype controls. Antibodies were tested alone, in all dual combinations and using a cocktail of all six antibodies. Intracellular cytokine staining was used to determine production of CD107a, IFNγ, TNFα and IL-2 in total and subsets of CD4+ and CD8+ T cells. Fold changes (FC) for IC antibodies compared to isotype controls were obtained. Bliss independence model was used to determine if the effect was synergistic.

Results: We detected a significant increase in the percentage of cells expressing IFNγ and TNFα but not IL-2 or CD107a following stimulation with gag and nef peptides. The addition of single IC antibodies or all six together led to minimal change in cytokine production. In contrast, we observed a statistically significant increase in the percentage of gag-specific cells expressing CD107a in the presence of anti-LAG-3 combined with anti-TIGIT (median fold change, FC compared to isotype controls 1.75x) and anti-CTLA-4 (median FC 1.38x) in HIV-specific CD4+ T cells. We also observed increased frequency of cells expressing IL-2 (median FC 1.26 – 2.17x) with combinations of CTLA-4, TIGIT, TIM-3, PD-L1 and LAG-3, in both HIV-specific CD4+ and CD8+ T cells. The largest FC increase in IL-2 was observed when anti-CTLA-4 was combined with any other antibodies, except anti-PD1.

Conclusion: Multiples combinations of two IC antibodies (including LAG-3, CTLA-4 or TIGIT) can enhance the frequency of polyfunctional HIV-specific T cells ex vivo in samples obtained from PWH on suppressive ART. The increased production of IL-2 with dual IC blockage could have a significant functional effect on both proliferation and cytotoxicity. Given the better safety profile of anti-LAG-3 and anti-TIGIT, these novel antibodies should be further explored in strategies to control HIV in the absence of ART.

281 SELECTIVE DEPLETION OF TIGIT-EXPRESSING MEMORY HIV-SPECIFIC CD8+ T CELLS IN HIV-1 AND cART

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Background: The expression of inhibitory Receptors (IRs) blocks CD8+ T-cell activity in HIV-1 infection. Consequently, the control of IRs is critical for recovering CD8+ T-cell function. However, the alteration of IR expression by HIV-1 infection is not fully understood and essential to identify future immunotherapeutic targets.

Methods: With this aim, we selected PBMCs from early (Ei, n=24) and 10 years (S10) on suppressive ART. For comparison, we selected healthy controls (HC, n=24). We performed cytofluorimetric analyses using IRs (TIGIT, PD-1, LAG-3 TIM-3 and CD25), functional (CD107a, IFNγ and IL-2) and lineage markers (CD3, CD4, CD8, CD45RA,CCR7 and CD27) in basal, SEB and HIV-1 conditions. Moreover, we analyzed multivariate datasets using Flowcytometry, R packages and we compared classical and unsupervised net-SNE single-cell analysis. We also evaluated IR candidates by short-term antibody blockade in CD8+.

Results: Our data revealed the expansion of TIGIT in CM and TM CD8+ during HIV-1 (p<0.05). We observed a negative correlation between CD4+ counts and TIGIT expression in CD8+ (p<0.05, r=-0.58). Single-cell analyses further
delineated the increase of three differential clusters of CD8+s (p<0.05, HC vs HIV-1) sharing effector and memory-like features together with TIGIT and TIM-3 expression. Also, single-cell analysis identified six differential clusters in response to SEB and five in HIV-1. These clusters decreased in frequency in HIV-1 infection and CART sharing memory and effector-like features, TIGIT expression and functional heterogeneity. Complementary to this, we observed a decrease of HIV-specific TIGIT CM CD8+s producing CD107a (Ei vs S2, p<0.05) and a depletion in response to SEB of TIGIT+TIM-3 Effector CD8+s producing CD107a (HC vs S2, p<0.05). Besides, TIGIT CM CD8+s with production of IFNy expanded upon SEB in CART (HC vs S2, p<0.05). Short-term antibody blockade of TIGIT and TIGIT+TIM-3 favoured the recovery of CD107a degranulation in CD8+s.

Conclusion: Our data point towards irreversible alterations of TIGIT expression in CD8+s with HIV-1 infection despite CART. These alterations were driven by the depletion of specific cellular clusters of CM and Effector CD8+s associated with antigen specificity and a loss of degranulation potential. We propose the targeting of TIGIT to recover degranulation activity in CD8+s.

282 SIGLEC-9 DEFINES AND RESTRAINTS AN NK SUBPOPULATION HIGHLY CYTOTOXIC TO HIV+ CELLS

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Background: Siglec-9 is an MHC-independent inhibitory receptor selectively expressed on a subset of the cytotoxic CD56dim natural killer (NK) cells. Siglec-9 restrains NK cytotoxicity by binding to sialic acid glycans on the surface of target cells. Despite the importance of such Siglec-9 interactions in tumor immune evasion, their role during HIV infection has never been investigated.

Methods: We phenotypically characterized Siglec-9+ CD56dim NK cells from 45 donors: 10 HIV-negative controls; 11 HIV+ viremic; and 24 HIV+ on suppressive antiretroviral therapy (ART), using multiparametric cytometric analysis. We measured total HIV DNA in CD4+ T cells by qPCR. Next, we examined the functional ability of total, Siglec-9+, Siglec-9-depleted, and Siglec-9- CD56dim NK cells (isolated from PBMCs of 3-6 healthy donors) to degranulate and kill cell lines (HUT78 and CEM.NKR) infected with HIV in the presence or absence of in-house Siglec-9 blocking antibody. Degranulation was measured as CD107a and IFNγ co-expression on NK. Killing was evaluated by lactate dehydrogenase release and CFSE/SYTOX Red assays.

Results: Our phenotypic analysis showed that Siglec-9+ CD56dim NK frequency is 1) decreased during viremic HIV infection and remains decreased despite ART; and 2) inversely correlated with levels of CD4+ T cell-associated HIV DNA during ART (P<0.01). During viremic and ART-suppressed HIV infection, Siglec-9+ CD56dim NK cells exhibit an activated phenotype with higher frequencies of NK activating/cytotoxic receptors and markers (NKp30, CD38, CD16, DNAM-1, perforin) and lower expression of the inhibitory receptor NKG2A, compared to Siglec-9- CD56dim NK cells. We observed increased frequencies of CD56dim NK cells in CD8+s with HIV-1 infection despite cART. These alterations were driven by the depletion of specific cellular clusters of CM and Effector CD8+s associated with antigen specificity and a loss of degranulation potential. We propose the targeting of TIGIT to recover degranulation activity in CD8+s.

Conclusion: Our data support a model in which Siglec-9+ CD56dim NK

283 PROVIRAL BURDEN, GENETIC DIVERSITY, AND DYNAMICS DURING ART IN VIREMIC CONTROLLERS

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Background: Viremic controllers can yield insights into HIV persistence, but they are underrepresented in reservoir dynamics studies. We combined single-genome sequencing (SGS), proviral quantification, phylogenetics and mathematical modeling to: i) reconstruct within-host pre-ART HIV evolutionary histories; ii) measure proviral burden, age and diversity on-ART; and iii) estimate proviral half-lives in 4 viremic controllers.

Methods: Three participants broadly maintained pVL <2000, while one eventually lost control, prior to initiating ART in chronic infection. We performed subgenomic HIV RNA SGS (n=1) on a median of 12 longitudinal pre-ART plasma samples/participant. Two PBMC samples from a median of 1.1 and 1.9 years on ART were also analyzed: the first for reservoir quantification using the Intact Proviral DNA Assay (IPDA); and the second for proviral nef SGS. Proviral sequence ages were inferred using a phylogenetic approach that leverages within-host pre-ART HIV evolutionary rates; we then applied a published mathematical model of reservoir seeding and decay to infer host-specific proviral half-lives from these data.

Results: We collected 356 unique plasma HIV RNA sequences (range 52-173/patient) and 206 intact, non-hypermutated, unique proviral sequences (12-118/patient). All within-host phylogenies exhibited molecular clock signal pre-ART (range 1.16c:10-5-5.35c:10-5 substitutions/base/day). Pre-ART pVL area under the curve correlated strongly with longitudinal pre-ART plasma HIV sequence diversity, total on-ART proviral burden and overall on-ART proviral diversity (all Spearman’s r=1; p<0.001). Total proviral %, quantified by IPDA, ranged from 9-94%, where the latter was observed in the individual who eventually lost control prior to ART. For two participants, inferred proviral integration dates ranged from shortly following infection to CART initiation; for the other two, the participating who lost control, proviruses largely dated well into chronic infection. For three of these participants, the best-fit proviral half-life estimates were <1 year, suggesting relatively rapid proviral turnover pre-ART; the fourth’s proviral pool was consistent with negligible decay following deposition.

Conclusion: Despite their viremic control, significant within-host pre-ART HIV evolution nevertheless gave rise to diverse within-host proviral pools with varying intact genome burden. HIV eradication strategies must overcome within- and between-host diversity in proviral landscape.
HIV INTEGRATION INTO BACH2 AND STAT5B IS PREVALENT EARLY IN INFECTION

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Background: Persistence of HIV despite ART remains a barrier to a cure. It is known that HIV proviruses are overrepresented in the BACH2 and STAT5B genes in persisting cells. Also, 9% and 31%, respectively, of individuals in chronic HIV infection have been reported to produce hybrid transcripts initiating in the HIV-LTR and spliced upstream of the gene start codon, thus subverting their regulation.

Methods: Single-copy sensitive nested PCR was used to identify hybrid transcripts in negatively selected CD4 cells from 44 individuals from the SABES/MERLIN primary infection cohort. This cohort enrolled uninfected MSM and transgender women in Lima, Peru between 2013 and 2015 and followed them for monthly HIV testing (Ab and RNA). Infected individuals started ART within 9 months of HIV acquisition. Hybrid transcripts were examined longitudinally for up to 4 years.

Results: A total of 179 samples with a median of 1.6x10^6 CD4 cells (range 3.3x10^5 – 3.4x10^6) per sample were examined. 4,490 nested PCRs were performed, with the transcript structure of all 394 positive reactions confirmed by sequencing. In total, 19 of 44 individuals (43%) had detectable BACH2 hybrid transcripts and 30 of 44 (68%) had STAT5B hybrid transcripts, with 17 of 44 (39%) positive for both. In most cases, hybrid transcripts were detected at dilution endpoint (~4 copies per million CD4 cells). Given that each cell likely produces a large number of transcripts, the infected cell population with proviruses at STAT5B and BACH2 was likely much lower. Despite the strong stochastic component for positivity at such low levels, once hybrid transcripts were observed they were often found in later samplings (11/15 for BACH2 and 17/24 for STAT5B), indicating that proliferation maintained these cells in the ART-treated individuals. Finally, the position and orientation requirements for hybrid transcripts detection and that all participants were treated early in infection, these data represent a significant underestimation of the fraction of HIV-infected individuals with HIV integrations in BACH2 and STAT5B.

Conclusion: HIV integration into BACH2 and STAT5B occurs in a large fraction of individuals early in infection. Cells producing hybrid transcripts are very likely to be maintained, albeit at low levels, by cell proliferation. Given that both genes are important regulators of T cell function, including T regulatory cell function, targeting these cells for elimination may be important for HIV cure strategies.

LONGITUDINAL ANALYSIS OF RESERVOIR DYNAMICS IN SIV-INFECTED MACAQUES ON LONG-TERM ART

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Background: Rhesus macaques (RMs) infected with SIV are a critical model for HIV-1 infection of humans. Previous studies suggest the SIV latent reservoir stabilizes between 36-60 weeks on ART at a frequency of intact proviruses 1-2 logs higher than in people living with HIV-1 (PLWH). Similarly, full-genome sequencing indicates that APOBEC-mediated hypermutation is both more frequent and extensive and that intact proviruses represent a greater proportion of the reservoir. Longitudinal analysis of the frequency of intact and defective proviruses in PLWH has revealed differential decay kinetics of these 2 populations and points to potential mechanisms that contribute to reservoir persistence. However, analogous studies in SIV-infected macaques have been hampered by the short duration of ART and lack of methods to quantify defective genomes. It is therefore unclear how the size and composition of the SIV latent reservoir in RMs on long-term ART compares to the HIV-1 latent reservoir in humans.

Methods: Using the SIV intact proviral DNA assay and a novel assay to quantify hypermutated viral genomes we describe the first longitudinal analysis of the size and composition of the SIV latent reservoir in a cohort of 10 RMs on ART for >2 years. With this set of assays, we quantify the frequency of different types of viral DNA spanning 8 weeks to nearly 3 years on therapy and use this to determine the decay rate of each population.

Results: We found that the half-life of intact proviruses in SIV-infected RMs during the first 2 years on ART is approximately 12 months — much shorter than the 44 months described for the HIV-1 latent reservoir. Hypermutated proviruses also decay but with a half-life of 29 months.

Conclusion: Our data shows that the SIV latent reservoir composition and size continue to change beyond the 1st year on ART — challenging previous assumptions that this pool stabilizes between 6 months and 1 year of therapy. The more rapid decay of intact relative to defective proviruses is consistent with observations in PLWH. Furthermore, the accelerated rate of decay for both types of proviruses relative to similar studies in PLWH suggests mechanisms underlying viral persistence may differ between SIV and HIV-1. These results provide essential benchmarks for researchers to evaluate the efficacy of interventions aimed at reducing the size of the latent reservoir in the SIV model and indicate that future studies must account for the decay that continues without interventions after the first year on ART.

DYNAMICS OF INTACT PROVIRAL SEQUENCES IN EARLY TREATED HIV-1 CLADE C–INFECTED INFANTS

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Background: Understanding the mechanisms that allow HIV to persist long-term is important for advancing HIV cure research, specifically in infants who acquired HIV perinatally. We sought to evaluate longitudinally the genetic composition of the proviral reservoir in infants perinatally infected with HIV-1 Clade C.

Methods: We assessed the proviral reservoir in 6 infants from Mozambique who initiated ART within the first 2 months of life and were followed for a median of 23 (IQR 22-23.25) months. We used Full Length Individual Proviral Sequencing (FLP-Seq) to evaluate the relative frequency and clonality of intact and defective proviruses over time.

Results: From the 6 infants, we obtained a cumulative total of 22 intact (11%) and 186 defective (89%) proviral sequences generated from a cumulative total of 51 million PBMCs over time. Four out of the 6 infants achieved virologic suppression (<200 HIV RNA copies/mL) following ART initiation and maintained viral suppression for the entire study period, while 2 of the infants experienced a transient loss of viral control at 8- and 11-months post ART initiation. We observed a gradual decline of total HIV copies during the observation period ranging from a median of 8.52 (IQR 4.39-36.50) HIV copies/10^6 PBMCs at the first sampling timepoint down to a median of 1.08 (IQR 0.28-3.12) HIV copies/10^6 PBMCs at the last sampling timepoint. Interestingly, this decline of HIV copies was more pronounced in intact proviruses which declined from a median of 0.72 (IQR 0-17.29) HIV copies/10^6 PBMCs at the first sampling timepoint to no detection at the last sampling timepoint. In one of the infants, we detected 4 clonal intact proviruses over 4 months post ART initiation and we also detected 4 members of the same clone 13 months later, indicating persistence of clonally expanded intact proviral sequences over a long period of time. In between these 2 time points, this infant displayed a short episode of rebound viremia of over 100, 000 HIV RNA copies/mL. However, this rebound viremia did not affect the detectable composition of the intact proviral reservoir.

Conclusion: We observed a faster decline of intact proviruses in these infants, suggesting an increased vulnerability of intact proviral sequences to antiviral immune effects. Additionally, we observed clonal expansion of intact proviruses at early stages in pediatric HIV infection consistent with an important role of clonal proliferation of virally infected cells for virus reservoir homeostasis and maintenance.

CYTOKINE DYSREGULATION AND ANTIGEN RESPONSES DRIVE T-CELL EXPANSION IN HIV INFECTION

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Background: Despite effective antiretroviral therapy (ART), HIV persists in CD4+ T cells which are maintained by clonal expansion. Even in virally
suppressed individuals, HIV infection induces persistent immune dysfunction. Individuals receiving immediate ART have smaller size of HIV latent reservoir and lower levels of chronic immune activation. However, most individuals seek medical attention after 6 months of infection. We propose to identify drivers of T cell clonal expansion and immune dysfunction in HIV-infected individuals receiving delayed (versus immediate) ART as therapeutic targets.

**Methods:** From the SABES Study in which HIV infection was prospectively tested monthly, we obtained paired blood samples (during acute infection and after one year of suppressive ART) from 6 HIV-infected individuals (3 receiving immediate ART within 2 months of infection, 3 receiving delayed ART 6 months after diagnosis). Using single-cell ECTESTseq, we captured surface protein expression, transcriptome, HIV RNA, and T cell receptor sequences in the same single cells. We used machine learning algorithms to identify the impact of delayed ART on the gene expression profile of CD4+ T cells, determinants of T cell clonal size, and markers differentiating clones containing HIV-RNA+ cells.

**Results:** We captured the single-cell multi-omics landscape of a total of 122,685 single cells (~8,179 cells per sample). Among them, we identified a total of 90 HIV-infected cells and 19 expanded CD4+ T cells harboring HIV-RNA+ cells. We found that interferon (IFN) responses are upregulated during viremia and returned to baseline after viral suppression, while T cell activation, immune exhaustion, and tumor necrosis factor (TNF) responses persisted despite one year of viral suppression in both immediate and delayed ART. Delayed ART upregulates cytokine regulation related genes such as ZFP36, DUSP2, and BHLHE40. While the major determinant of T cell clonal size is antigen-response-genes, the regulators of T cell clonal size in delayed ART are immune exhaustion, IFNγ, and TGFβ responses. Finally, HIV-RNA+ cells are enriched in proliferating Th1 effector cells.

**Conclusion:** We found that delayed ART induces persistent immune activation and dysregulated cytokine responses. HIV persists in Th1 effectors that proliferate secondary to persistent immune activation. Our study suggests that reducing chronic HIV antigen stimulation and cytokine dysregulation may potentially reduce the clonal expansion of HIV-infected cells.

**288 IMPACT OF REPRODUCTIVE AGING ON HIV PERSISTENCE IN CISGENDER MEN AND WOMEN WITH HIV**

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**Background:** Women represent the majority of HIV infections, yet sex differences in HIV reservoir dynamics during reproductive aging remain an under-explored area of research.

**Methods:** Longitudinal samples from virally suppressed cis-gender women (N=60, 285 samples) and men (N=31, 130 samples) were retrospectively identified from the AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT) population. Participants were between the ages of 40-53 at the time of ART initiation and did not take hormonal therapy during analytic period. At each timepoint, levels of estradiol were measured by ELISA, cellular HIV DNA (total) and HIV RNA (unspliced and tatrev) were quantified by droplet digital PCR (ddPCR). Inducible HIV RNA was quantified on a subset of 132 samples from 11 participants by EDTIS (measuring cell associated env mRNA after induction by TCR stimulation). We used mixed-effects model with a random participant intercept including normalized outcomes (total HIV DNA, HIV RNA and inducible HIV RNA) and sex, time since ART initiation, and the sex by time interaction as predictors.

**Results:** At baseline, median (IQR) CD4+ were 219 (82,324) cells/ul for women and 248 (120, 290) for men. Median age (IQR) was 45 (42,48) and 47 (43,51). Median follow up (IQR) was 93 (76,132) and 74 (52,93) months. As expected, levels of estradiol decreased among female participants (p<0.01). Overall, we observed a significant decline of total HIV DNA over time in both men and women (p<0.01). However, the rate of change significantly differed between sexes (p<0.01) with women having a significantly slower rate of decline as compared to men which becomes more pronounced with age (Figure 1). The levels of inducible HIV RNA increased over time in women but not in men during reproductive aging. We did not observe a difference in the dynamic of cell-associated HIV RNA measures in the absence of ex vivo stimulation between sexes (p-values >0.17).

**Conclusion:** Previous work has demonstrated that the estrogen receptor is critical for the maintenance of HIV latency, but the intersection between aging and declining sex hormones is less clear. These studies demonstrate a sex specific HIV reservoir dynamic. While total HIV DNA (including intact and defective genomes) declines more slowly in women than in men, the inducible reservoir (enriched in replication competent virus) increases in women after menopause. The divergent behavior of the reservoirs in both sexes is an important parameter to be considered in cure trials.

![Image](https://via.placeholder.com/150)

**Figure Legend:** Longitudinal dynamic of total HIV DNA (10^9 CD4+ cells) (Log10). Thin black lines represent individual participants. The thick blue lines and shaded regions indicate model-derived predicted values and their 95% confidence intervals.

**289 SEX DIFFERENTIAL EXPRESSION OF IL-7 AND MARKERS OF HOMEOSTATIC PROLIFERATION**

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**Background:** Reproductive age women have lower levels of residual virus activity with antiretroviral therapy, along with lower levels of T cell activation and PD-1 expression. The estrogen receptor is a regulator of latency reversal. T cell correlates of reservoir size and activity differ in men and women. We sought to assess the cytokine environment in matched men and women on suppressive ART to identify features of residual immune activation that may impact reservoir maintenance.

**Methods:** Plasma samples from a previously described matched cohort of males and females with HIV infection on suppressive ART (n=26F, 26M) were analyzed using the Human Cytokine-30plex kit on the Mesoscale discovery multiplex platform. Analyses were assessed for relationship with reservoir size, activity, and T cell immune phenotype in men and women.

**Results:** Bivariate analysis identified 7 analytes with statistically significant sex-differential expression between males and females which included: IL-12, IL-17A, IL-6, IL-7, CCL17, IL18, and VEGF. Principle component analysis favored expression of IL-6 and IL-12 in females and the remainder in males. Of these analytes, IL-7 expression was also linked to history of AIDS as defined by CD4 nadir of <200. Plasma IL-7 levels were lower in women (p=0.004) and particularly among women with a CD4 nadir<200. IL-7 levels were positively correlated with CD4+HLADR+CD38+ (Pearson’s r=0.46, p=0.01) in females but not in males. CD8+HLADR+CD38+ had a similar relationship that did not reach statistical significance (Pearson’s r=0.35, p=0.080). There were no relationships between reservoir parameters (integrated and total HIV DNA, cell-associated HIV RNA, residual HIV viremia by single copy assay) and IL-7 in either sex.

**Conclusion:** Cytokine profile in ART-suppressed individuals identified lower expression of IL-7 among females, most pronounced when the CD4 nadir was <200. IL-7 expression was correlated with the CD4+HLADR+ single-positive population associated with homeostatic proliferation in women, but not men. Declining estrogen levels post menopause have been linked to changes in IL-7 expression. These results suggest that homeostatic proliferation may have a differential contribution to reservoir maintenance in females and males. Further studies are needed to define whether these relationships changes with estrogen decline during reproductive aging.

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290 TYPE I INTERFERON SIGNALING INDUCES HIV-1 LATENCY IN MACROPHAGES

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Background: The major obstacle to HIV cure is the existence of latent viral reservoirs – T cells and macrophages that harbor replication-competent, transcriptionally-silent proviruses. There remain crucial gaps in our understanding of the molecular mechanisms that lead to latent infection in macrophages. Prior studies from our laboratory have shown that interactions between macrophages and microbes that lead to type I interferon (IFN) production repress HIV-1 transcription, suggesting a central role for type I IFNs in establishing latent infection. We hypothesize that type I IFN signaling induces a state of transcriptional latency in HIV-1 infected macrophages by altering transcription factor recruitment to the viral promoter.

Methods: We examined HIV-1 replication kinetics and the effects of type I IFN signaling on HIV-1 replication in an in vitro monocyte-derived macrophage (MDM) model that employs a reporter virus encoding nanoluciferase under the control of the 5' LTR. Transcription factor recruitment to the 5' LTR was evaluated using chromatin immunoprecipitation (ChIP). Single cell RNA sequencing (scRNA-Seq) was utilized to determine changes in gene expression in infected macrophages.

Results: We show that HIV-1 replication peaked early after infection in MDMs and steadily decreased over time. This decrease correlated with decreased transcription, suggesting that HIV-1 enters a latent state. This transition to latency was associated with decreased recruitment of NF-kB p65 and RNA polymerase II to the 5' LTR. Comparing productively-infected MDMs to latently-infected MDMs using scRNA-Seq revealed differential expression of a number of IFN-regulated genes (IRGs). Blocking type I IFN signaling partially reversed the decrease in HIV-1 expression, suggesting that type I IFNs produced by infected MDMs contribute to the repression of viral replication. Furthermore, treating infected MDMs with type I IFNs led to a pronounced and sustained decrease in virus replication that simulated latency. Finally, blocking type I IFN signaling partially restored the interaction between NF-kB p65 and RNA polymerase II with the 5' LTR.

Conclusion: Our data suggest that type I IFN signaling, directly or indirectly, alters transcription factor recruitment to the HIV-1 promoter to induce a state of latency. These findings identify a key signaling pathway involved in the establishment of HIV-1 latency and may uncover possible targets for preventing or reversing latency in this critical viral reservoir.

291 MODULATION OF HIV TRANSCRIPTION USING AN IN VITRO MACROPHAGE HIV-1 LATENCY MODEL

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Background: HIV-infected macrophages persist despite viral suppression and can contribute to viral rebound upon treatment interruption, yet little is known regarding the establishment and control of latency in this important HIV reservoir. Physiologically-relevant in vitro systems that provide a robust, quantitative model of latent infection and reactivation are required to investigate factors that govern latency in macrophages.

Methods: Primary human monocyte-derived macrophages (MDM) were infected in vitro with a GFP-HIV reporter virus and FACS sorted 7-days post-infection, to purify GFP- populations consisting of uninfected bystander and non-productively infected MDM. GFP- MDM were cultured for a further 9 days in media containing entry inhibitor enfuvirtide (T20), to prevent de novo infection, and potential latency modulating agents. Reactivation of HIV transcription was quantified by live cell fluorescent microscopy via expression of GFP in reactivated cells.

Results: Spontaneous reactivation of HIV transcription within MDM was observed in all donors with a linear rate of 0.22% ± 0.04% (mean ± SEM, n=10) GFP+ cells per day, slower than rates of HIV transcription following initial infection (0.91% ± 0.12% GFP+ cells per day), indicating the presence of a population of potentially latently infected macrophages. Reactivated MDM produced replication competent virus, demonstrated by infection of heterologous PBMC in co-culture and in a cell-free infection system. Polarization of MDM to either M1 or M2 significantly inhibited (p=0.03) or enhanced (p=0.02) HIV reactivation, respectively. HIV reactivation was increased in unpolarised MDM by latency reversing agents (LRAs) including PKC agonist, bryostatin-1, and HDAC inhibitor vorinostat; however, the LRA panobinostat did not elicit reactivation in this model.

Conclusion: We have developed a robust and quantitative model of latently infected primary MDM, which can be used to advance cure strategies targeting the latent HIV reservoir. Our data suggest the potential of MDM to harbour latent HIV infection and contribute to viral rebound. The modulation of reactivation rates by polarization and latency reversing agents suggests latent macrophage reservoirs are sensitive to local environments in vivo, and may be therapeutically modulated. Moreover, the mechanisms governing latency in macrophages may differ to those in CD4+ T cells, potentially requiring macrophage-specific strategies to target HIV in these cells.

292 NOVEL CRISPR SCREENS IDENTIFY A ROLE FOR CUL3 IN HIV-1 LATENCY MAINTENANCE

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Background: HIV-1 establishes long-lived latent reservoirs that present a barrier for virus eradication. One approach to reduce the latent reservoir is the use of latency reversal agents followed by the killing of virus-producing cells. However, this approach is limited by the inability to reactivate the majority of latent proviruses, highlighting the need for a greater understanding of the interplay of mechanisms involved in the maintenance of HIV-1 latency.

Methods: We initiated a multi-arm CRISPR screening approach in the J-Lat model of latent infection to gain a comprehensive representation of the pathways involved in HIV-1 latency. Upon CRISPR/Cas9-mediated knockout of a
latency maintenance gene, the provirus of the J-Lat cell line is reactivated. The screen also adapts a functional CRISPR screening methodology developed in the Emerman lab called HIV-CRISPR. The HIV-CRISPR vector is a lentiviral vector that encodes Cas9, a library of single guide RNAs (sgRNA), and two intact LTRs that can be mobilized to produce an HIV-CRISPR RNA containing the sgRNA of interest in the presence of the reactivated HIV-1 provirus. The HIV-CRISPR RNA is packaged in trans into the reactivated virion and released into the supernatant. Sequencing is then used to quantify the enriched sgRNAs in the released viruses serving as a direct readout to identify genes controlling HIV-1 latency maintenance. We investigated the mechanisms controlling the chromatin state of latent HIV-1 by developing a custom sgRNA library specifically targeting ~800 human genes involved in some aspect of epigenetic control of gene regulation or DNA modification.

Results: The screen involving knockout of the epigenetics genes identified known HIV-1 latency maintenance factors including BRD4 and KAT5. Our top gene hit in the screen was Cullin 3 (CUL3), which is involved in ubiquitination of target proteins, and novel to HIV-1 latency. Knockout of CUL3 in two different J-Lat clones leads to the release of HIV-1 from latency. Similar experiments in primary CD4+ T-cell models of HIV-1 latency are ongoing.

Conclusion: We developed a new high-throughput, combination latency HIV-CRISPR screen that is able to identify novel and known HIV-1 latency maintenance genes. Initial validation of the screen demonstrates that protein ubiquitination through the CUL3 pathway is important for HIV-1 latency maintenance. Further analysis and combining the results from additional screens will provide a more comprehensive view of HIV-1 latency.

293 SYNERGISTIC COMBINATIONS OF LATENCY-REVERSING AGENTS IDENTIFIED USING CRISPR Screens

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Background: HIV-1 persisting in a latent form in resting CD4+ T cells despite effective antiretroviral therapy is the major barrier to cure. A promising therapeutic approach known as “shock and kill” seeks to achieve cure by sequencially reactivating latency in infected cells and then promoting the killing of productively infected cells. To date, however, no single LRA has been shown to reduce latent reservoir size in infected individuals. Functional genetic screening, especially CRISPR/Cas9-based screening, provides a global unbiased approach to understand the molecular aspects of HIV-1 infection. However, few studies focused on systematically identifying LRA combinations that overcome the limitations of individual LRAs.

Methods: We established a polyvalent in vitro model for HIV-1 latency. HIV-1 gene expression can be re-induced in >90% of this population by TNF-α treatment as evidenced by GFP expression. To identify candidate drug targets that potentially synergize with existing LRAs, we performed genome-wide CRISPR screening followed by induction of HIV-1 gene expression in latently infected cells using a suboptimal dose of a selected LRA, and then sorted for GFP+ cells directly. A gene whose knockout leads to enhanced GFP expression may emerge as the candidate drug target for synergy with the stimulating LRA if inhibitors of its function exist.

Results: We tested this approach using the SMAC mimetic AZD5582, an inhibitor of the non-canonical NF-kappa b (nchNF-kb) pathway, as an LRA and identified HDAC2, a histone deacetylation complex blocked by some HDAC inhibitors and BRD2, part of the Bromodomain and Extra-Terminal motif (BET) protein family that are targeted by BET inhibitors. Using CD4+ T cells from individuals on antiretroviral therapy, we confirmed synergy between AZD5582 and several HDAC inhibitors and between AZD5582 and the BET inhibitor JQ1. Remarkably, a reciprocal screen using an HDAC inhibitor as an LRA identified nchNF-kb regulators, especially BIRC2, as a druggable synergistic candidate for use in combination with HDAC inhibition, confirming the validity of this approach.

Conclusion: Our studies provide novel insights into the roles of host factors in HIV-1 reactivation and validate a system for finding drug combinations for HIV-1 latency reversal.

294 ACTIVATING PKC-ε INDUCES HIV EXPRESSION WITH IMPROVED TOLERABILITY

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Background: Activation of latent HIV could increase elimination of infected cells by therapeutics that directly target infected cells or stimulate antiviral immunity, potentially leading to long-term remission or cure. Protein kinase C (PKC) agonists are highly effective at activating latent HIV in vitro. We previously found that platelet activation is associated with toxicity of novel PKC agonist C232A and that this critical safety liability is broadly associated with known classes of PKC agonists. We hypothesized that specific targeting of PKC isoforms abundant in T cells but not in platelets could improve tolerability of PKC agonists that activate HIV.

Methods: PKC isoform expression in T cells and platelets was measured by western blot to identify differentially expressed isoforms. Isoform-selective agonists were identified by testing compounds for individual PKC isoform translocation. HIV RNA was assessed after treatment in cells isolated from ART-suppressed people living with HIV. In latently infected Jurkat cells, HIV induction was assessed by flow cytometry after expression of constitutively active PKC isoforms. In vivo platelet activation was assessed by hematology counts and T cell activation by EGR1 and CD69 mRNA quantification from whole blood in rats.

Results: Prostratin induction of HIV was unaffected by G6976, an inhibitor of classical PKC isoforms (PKC-а/β/γ), indicating that novel PKC isoforms (PKC-δ/ε/η) are sufficient for HIV activation. Expression analysis revealed high levels of PKC-δ and ε in platelets and T cells while PKC-β and α were only abundant in T cells. In Jurkat cells, PKC-ε was sufficient for HIV activation, supporting selective targeting of PKC-ε to reduce toxicity. Systematic modification of C232A led to the identification of C233, a novel PKC agonist 2-fold and 10-fold more selective for PKC-ε over PKC-δ, respectively. In rats, C233 and C232A increased Egr1 mRNA associated with T cell activation to similar levels, but C232A affected platelet levels more severely than C233. One of 6 animals treated with C232A was euthanized, but no deaths occurred with C233.

Conclusion: Platelet activation is a critical safety liability associated with non-selective PKC agonists and should be carefully monitored in preclinical and clinical studies. These results indicate that specifically activating PKC-ε might improve safety in vivo and support continuing structure-based design of selective novel PKC agonists for safe activation of HIV reservoirs.
295 VENETOCLAX SIGNIFICANTLY REDUCES HIV VIRAL LOAD IN VIVO AND IN VITRO
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Background: The BCL-2 pro-survival protein has been implicated in HIV persistence, and we have previously demonstrated that the clinically used BCL-2 inhibitor Venetoclax augments HIV induced killing of the reactivating cell, in vitro. It is unknown whether Venetoclax impacts HIV dynamics in vivo. We hypothesized that Venetoclax would reduce HIV replication in a murine model of HIV, and increase clearance of HIV infected cells by cytotoxic T Cells.
Methods: Primary CD4 T-Cells isolated from HIV infected, ART suppressed, donors were infected in vitro with HIV IIIB, subsequently treated with the BCL-2-specific inhibitor Venetoclax, and co-cultured with HIV Pemmix expanded, CD8 T Cells, in the presence of ART. The CD8-negative target cells were analyzed by flow cytometry to measure cell death by Live/Dead staining and intracellular p24 expression. Supernatant p24 was measured by ELISA to estimate viral production. Using NOD/Shi-scid/IL2Rγnull immunodeficient (NCG) mice, humanized using CD34+ cells isolated from human cord blood, we treated HIV infected CD34+ cell humanized mice with or without Venetoclax and assessed viral dynamics in the absence of ART, measured by HIV RNA PCR.
Results: Treatment of acutely HIV-infected CD4 T-Cells with the BCL-2 inhibitor Venetoclax significantly increased susceptibility to Cytotoxic T lymphocyte killing at E:T ratios of 1:2 and 1:5 (p <0.05), accompanied by significant reductions in levels of supernatant p24 (p <0.05). Within our in-vivo mouse model, BCL-2 inhibition resulted in significant, log-fold decreases in HIV RNA with Venetoclax (p = 0.002), accompanied by significant decreases in CD4 cells (p <0.0001) compared to control.
Conclusion: Taken together, these findings suggest that Venetoclax augments host immune function against HIV, in the setting of active HIV infection, both in an in-vitro and in-vivo setting. These findings represent a significant step forward in our understanding of the clinical applicability and feasibility of BCL-2 inhibition for HIV therapy.

296 AUTOLOGOUS VIRUS-NEUTRALIZING ANTIBODIES DELAY VIRUS REBOUND IN INFANT SHIV MODEL
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Background: Early ART improves disease outcomes in infected children but does not eliminate latent HIV reservoirs. Studies in a small subset of perinatally infected children who developed HIV-specific antibody responses and remained virus free when ART was interrupted, suggested that autologous antibody responses may be important for sustained viral control and lack of virus rebound. However, kinetics of HIV-specific antibody responses and their impact on virus rebound in the setting of postnatal HIV transmission is unclear. We used an established infant rhesus macaque (RM) SHIV infection model with delayed ART to define the kinetics, specificity, breadth, and antiviral functions of HIV-specific antibodies in the setting of postnatal HIV infection.
Methods: 10 infant RMs were orally challenged with SHIV.C.CH555 3755 dCT and daily triple ART initiated at 8wpi. ART was interrupted after 52 weeks and virus rebound was monitored by viral RNA detection in infant plasma. Antibody development was assessed by binding to the autologous virus CH555 TF gp120 and MN gp41. HIV epitope and clade specificities were assessed using a multiplex luminescent assay (BAMA). Plasma antibody neutralizing and non-neutralizing functions were evaluated at time points pre-ART, during ART, and at TI.
Results: HIV envelope (Env) gp120- and gp41-specific antibodies were first detected at 4wpi and decline after ART initiation (8wpi). Yet, the antibody levels remained detectable throughout ART. Plasma Env-specific antibodies predominantly targeted the V3-loop, C5 region, and showed broad reactivity against heterologous HIV EnvS. Plasma antibody at pre-ART, during ART, and at post-ART mediated robust ADCP activity against CH555 TF- gp120-coated beads but limited ADCC activity against SHIV-infected cells at similar time points. 5 of 10 infants developed autologous virus neutralization while on ART. Upon ART, virus rebound was observed in all infants (range, 7-35 days) except 1 infant that developed highest level of autologous plasma neutralization. Pseudoviruses generated using rebound EnvS were resistant to autologous plasma neutralization. Computational machine learning analyses identified autologous neutralization as strongest predictor of delayed virus rebound.
Conclusion: Development of autologous virus neutralizing antibody may delay time to virus rebound. This study underscores the importance to explore augmentation of autologous virus neutralization as a potential strategy to afford viral remission or cure in pediatric HIV.

297 VIRUS REMISSION WITH AN OPTIMIZED EARLY ART REGIMEN IN MACAQUES
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Background: Early initiation of antiretroviral therapy (ART) does not cure HIV in humans or SIV in macaques due to rapid viral reservoir establishment. Here, we investigated in macaques if a novel ART regimen, designed to have robust penetration in virus reservoir sites, can result in virus remission after treatment cessation.
Methods: The ART regimen included daily tenofovir alafenamide (TAF; 1.5 mg/kg), and emtricitabine (FTC; 20 mg/kg), and monthly rilpivirine long-acting (RPV LA; 200 mg/kg), and cabotegravir long-acting (CAB LA; 50 mg/kg) for 6 months followed by 6 months of CAB LA/RPV LA maintenance therapy. TAF was selected to enhance tenofovir-diphosphate (TFV-DP) levels in lymphoid tissues (LT), CAB LA and RPV both distribute in the central nervous system. TAF/FTC were given at human-equivalent doses and administered orally to mimic drug biodistribution in humans. CAB LA/RPV LA were given intramuscularly at doses that maintain human therapeutic drug levels. Macaques were infected intrarectally with RT-SHIV and initiated treatment at day 5-6 post infection (n=4) or were untreated (n=2). SHIV RNA in plasma was monitored by RT-PCR (LLOQ=12.5 copies/ml) during 1 year of ART and a 20-month period of no treatment which included in vivo CD8+ cell depletion with monoclonal antibody MT07871 at month 16. Drug concentrations were measured by HPLC-MS/MS.
Results: Peak viremia in treated animals was 3.4 (range=2.7-4.3) log10 RNA copies/ml compared to 6.8-7.0 in untreated controls. Treated macaques achieved viral suppression 14-22 days after treatment initiation and remained aviremic during 1 year of ART and the 20-month follow-up period. Median plasma CAB levels during treatment were 2.11 ug/ml and became undetectable 3 months after the last dose. RPV testing is ongoing. Median concentrations of TFV-DP in PBMCs, LT, and RT were 1427 and 367 fmol/106 cells, and 14.6 fmol of mg, respectively. Median FTC-TIP levels were 498 and 260 fmol/106 cells in PBMCs and LT, respectively, and were undetectable in RT. Treatment with MT07871 effectively depleted >99.9% of CD8 + T cells in blood. Plasma SHIV RNA remained undetectable during and 4 months after CD8 depletion.
Conclusion: We identified in macaques a suppressive ART regimen that results in virus remission following early treatment. The lack of viral rebound 20 months after treatment cessation and CD8 depletion is encouraging and highlights the need to further define the effect of optimized early ART regimens on virus remission.

298 EFFECT OF RECOMBINANT GROWTH HORMONE ON HIV RESERVOIRS: A PILOT STUDY (CTN 298)
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Background: Administration of recombinant human growth hormone (rhGH) in ART-treated individuals has been shown to increase thymic output and CD4+ T cell counts. We hypothesized that the production of naive T cells induced by rhGH may lead to the clearance of infected memory CD4+ T cells by repopulating the CD4+ T cell niche.
Methods: Twelve HIV-1-infected adults (<40 years of age) on stable ART were enrolled in an open-label single-arm study of rhGH therapy. rhGH was administered by subcutaneous injection on an outpatient basis for a total of 48 weeks (3 mg/day for 24 weeks, followed by 1.5 mg/day for 24 weeks). PBMCs
were collected at baseline and every 12 weeks. In isolated CD4+ T cells, we measured thymic output [T Cell Receptor Excision Circles (TRECs)] quantification by qPCR, as well as the size of the HIV reservoir by HIV DNA qPCR, tat/rev limiting dilution assay (TILDA) and a modified quantitative viral outgrowth assay (mQVOA).

Results: Most of the participants were male (10 males, 2 females), with a median age of 34 years and a median duration of ART of 3.7 years. No serious adverse events were reported. However, nine participants discontinued rTGH therapy before 48 weeks, most commonly due to musculoskeletal pain (n=6), which resolved after drug discontinuation. To assess the effects of rTGH, we compared baseline values to measures performed at the last visit on active drug and at which PBMCs were available (n=10, mean duration of rTGH therapy = 25 weeks). As expected, the frequency of TRECs in CD4+ T cells slightly increased (1.5 mean fold change; p<0.01), reflecting an increase in thymic output. However, absolute CD4 T cell counts remained unchanged. Administration of rTGH led to a modest but significant reduction in the frequency of CD4+ T cells harboring total HIV DNA (0.8 mean fold change; p=0.01). The frequency of CD4+ T cells with the ability to produce Tat/Rev transcripts upon stimulation remained stable. There was a trend for a decrease in the frequency of CD4+ T cells harboring replication competent HIV (mean fold reduction of 0.41 infectious units per million cells, p=0.08).

Conclusion: In this pilot study, administration of rTGH to individuals on ART led to a modest but statistically significant reduction in HIV reservoir markers despite early rTGH discontinuation in most participants. Reservoir reduction approaches based on a “fill to replace” strategy warrant further investigation.

299 EFFECTS OF PD-1 BLOCKADE ON HIV RESERVOIRS IN BLOOD DURING ART
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Background: Blockade of the programmed cell death protein-1 (PD-1) pathway can reverse HIV latency, can potentiate virus-specific CD8 T cell responses, and can reverse HIV latency, can potentiate virus-specific CD8 T cell responses. However, administration of checkpoint inhibitors is also associated with adverse outcomes such as the induction of antiviral T cell clonotypes that produce pro-inflammatory cytokines. Here, we evaluated the effects of PD-1 blockade on HIV reservoir size and drug specificity in individuals on ART.

Methods: 14 HIV-infected individuals were enrolled in a study of PD-1 blockade during ART. PBMCs were collected at baseline and at 3 and 24 weeks post-infusion. Control participants who had not received pembrolizumab were also studied. CD4 T cells in PBMC were FACs-sorted into naive, central/transitional memory (CTM), and effector memory (EM) subsets. HIV DNA in sorted cells was quantified by limiting dilution PCR of a portion of env. PCR products were Sanger sequenced. HIV RNA was quantified by qRT-PCR. Cell gene expression patterns were determined by mRNA-Seq. Paired integration site analysis and near-full length sequencing was performed by multiple-displacement amplification and single-genome sequencing. Intact HIV proviruses in total CD4 T cells were enumerated by intact proviral DNA assay. The expression of HIV-RNA transcripts was measured in 10 cell populations (CD4+, TN, TCM, TTM, TEM, TFH, TCDO2, TCDO3, and TCDO2high) by cell-specific probes. The expression of HIV-DNA and intact provirus were measured in all cell populations by ddPCR.

Results: Pembrolizumab infusion and 24 weeks of follow-up were completed for six participants. Changes in sorted cell HIV DNA and RNA over time were similar in pembrolizumab-treated and control groups. However, a shift in distribution of HIV-infected cells to the EM subset and a reduced genetic diversity of HIV DNA sequences were observed after pembrolizumab. These were associated with lower levels of T cell activation signaling gene transcripts and with expansion of a small number of HIV-infected cell clones. In one participant, one clone expanded to account for ~1% of all circulating CD4 T cells post-infusion. This clone harbored a provirus with a deletion in the major splice donor site. The provirus was infrequent in both pembrolizumab-treated and control participants, with no consistent change in frequency over time in either group.

Conclusion: PD-1 blockade was associated with perturbations in the HIV-infected CD4 T cell pool including a shift to an EM phenotype and further expansion of some infected cell clones. Further investigation of intact proviruses in infected T cell clones following checkpoint inhibitor administration will help clarify the net effect of these agents in HIV cure strategies.

300 THE PERIPHERAL CD4+ T-CELL RESERVOIR ATLAS IN cART-TREATED HIV-INFECTED INDIVIDUALS
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Background: The knowledge of the mechanisms that govern the persistence of the distinct subpopulations of CD4+ T cells harboring HIV could help to design new therapies to cure HIV. Here, we evaluate the distribution of the HIV reservoir in 12 different CD4+ T cell subpopulations in peripheral blood and its relationship with immune activation, cell proliferation and cytokine profiling.

Methods: A 500ml blood drown was obtained from 14 HIV-infected individuals under ART from Hospital Vall d’Hebron. From PBMCs, we isolated total CD4+ T cells, TN, TCM, TTM, TEM, TFH, TCDO2, TCDO3, and TCDO2high cells by cell sorting, and activated and resting CD4+ T cells using magnetic beads. Total HIV-DNA and intact provirus were measured in all cell populations by ddPCR. The expression of HIV-RNA transcripts was measured in 10 cell populations (CD4+, TN, TCM, TTM, TEM, TFH, TCDO2, TCDO3, and TCDO2high) by the RNA FISH/flow assay, and by the cell-associated HIV-RNA assay in total CD4 + T cells. Activation (CD69, HLA-DR and CD38), proliferation (Ki-67) and exhaustion (PD-1) cell markers were studied by flow cytometry. Ultrasensitive viral load (usVL) and cytokines (Luminex) were measured in all plasma samples.

Results: Total HIV-DNA showed that the most infected cell populations had a memory phenotype (TTM, TEM, TCD02high, TCDO2, and TEM), harboring all cell populations studied with intact provirus. Moreover, TCDO2, TFH, TEM, and TCDO2 were the main cell populations supporting HIV-1 transcription. We found significant positive correlations between HIV-DNA and HIV-RNA expression in 8/10 cell populations measured by ca-HIV-RNA, TCDO2 and TCDO3 measured by RNA FISH/Flow; and TEM, TFH and TCM measured by usVL. Phenotypic analysis by flow cytometry showed that cell populations with higher provirus were, in general, more exhausted and less activated (TTM, TEM, TCDO2high); and that cell populations with higher levels of proliferation had also a high HIV-RNA expression (TFH, TCDO2). HIV-DNA in TCM cells were negatively related with the plasma cytokines IFNγ, TNF and IL17a/IL17b, being the latest significantly correlated with HIV-DNA in 7/10 populations studied.

Conclusion: Upon simultaneous analysis of 12 CD4+ T subpopulations in peripheral blood, we found that the different cells that composed the persistent HIV reservoir during ART are linked to different activation and inflammation profiles, demonstrating the high heterogeneity of the HIV reservoir. This study provides new knowledge to target specific cell reservoirs.
301 HIV PERSISTS PREFERENTIALLY IN MEMORY CD4+ T CELLS CO-EXPRESSING PD1 AND CTLA4

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Background: Identifying cellular subsets that preferentially harbour HIV is important for curative strategies. We aimed to address the role of the immune checkpoints, programmed cell death 1 (PD1) and cytotoxic T-lymphocyte associated protein 4 (CTLA4) for HIV persistence on ART.

Methods: We collected peripheral blood mononuclear cells (PBMCs) and lymph node (LN) mononuclear cells by performing leukapheresis and LN biopsies in people with HIV (PWH) on suppressive ART. Memory CD4+ T cells were sorted into four subsets based on their expression of PD1 and CTLA4 to obtain: double-positive (PD1+ CTLA4+), PD1 single positive (PD1− CTLA4+), CTLA4 single positive (PD1− CTLA4+), and double-negative (PD1− CTLA4−) cells. Within each sorted subset from blood and LN we quantified total HIV DNA and cell-associated unspliced HIV RNA (CA-US HIV RNA) and also performed the tat/new limiting dilution assay (TILDA) to quantify the frequency of cells with inducible multiply-spliced HIV RNA.

Results: We enrolled 21 PWH with 4.2-14.1 years of ART-mediated viral suppression. We obtained paired LN biopsies and leukapheresis samples in 8 participants and leukapheresis only in 13 participants. The frequency of memory CD4+ T cells co-expressing PD1 and CTLA4 was higher in LN tissue compared to blood whereas double-negative cells were more frequent in blood. We found a significant enrichment of total HIV DNA in blood memory CD4+ T cells co-expressing PD1 and CTLA4 with a median 1.8-fold (IQR 1.1-2.5, P=0.018) higher level of HIV DNA when compared to their double-negative counterpart. This enrichment was not seen in LN cells. The frequency of cells containing HIV DNA within most PD1/CTLA4 subsets in both blood and LN correlated with higher CD8+ T cell counts and percentages at study entry. Despite their enrichment for total HIV DNA, a lower proportion of double-positive memory CD4+ T cells in blood produced multiply spliced HIV RNA upon PMA/ionomycin stimulation. There was no difference across PD1/CTLA4 subsets in the level of CA-US HIV RNA in blood or LN.

Conclusion: The frequency of HIV-infected cells was moderately higher in blood memory CD4+ T cells co-expressing PD1 and CTLA4 but this enrichment was not seen in LN. Double-positive memory CD4+ T cells from blood had a lower frequency of inducible virus, potentially indicating these cells are characterised by their negative signalling and a limited susceptibility to induction of latent HIV.

302 SINGLE-CELL TRANSCRIPTOMIC T-CELL STATES OF A RESERVOIR-MARKING HU-MOUSE MODEL

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Background: Human immunodeficiency virus (HIV-1) causes a chronic infection in which the virus persists in a latent state in patient CD4+ T cells integrated into the host genome. This pool of virus persists during effective antiretroviral therapy (ART) and represents the major barrier to cure. Our ability to study the HIV-1 reservoir is limited because current model systems require activation of latently infected cells to enumerate or characterize them. The activation of the cells disturbs the unique characteristics that maintain latency.

Methods: We have developed a novel HIV-1-induced lineage tracing (HILT) humanized mouse model that can irreversibly report if a CD4 T cell was ever infected by HIV-1. The system utilizes a genetically encoded, cre-lox activated fluorescent protein switch in which a cre-expressing virus irreversibly changes the phenotype of target cells that are transduced as stem cells to express the cre-activated switch. We validate the system and examine HILT-marked cells using single cell RNA sequencing to provide transcriptional profiles of HIV-infected cells before and after antiretroviral therapy (ART).

Results: The HILT model recapitulates features of HIV-1 pathogenesis including sustained viremia, CD4 cell depletion, response to ART and re-emergence of virus following treatment interruption. Using high throughput single cell RNA sequencing (scRNAseq) we have obtained transcriptional profiles of acutely infected and persistently infected CD4 T cells following the initiation of ART. Splenic CD4 T cells are organized in 7-8 major transcriptionally defined clusters and acutely infected and persistently HIV-1 infected cells are distributed in diverse subsets indicating that HIV infects and persists in cells with diverse transcriptional states. During acute infection HIV gene expression is detectable, whereas following ART, viral mRNA was not detected in cells.

Conclusion: HIV infected cells are interspersed in diverse clusters of CD4 T cells, both before and after ART. HIV-associated differential gene expression analysis reveals pathways that with known interactions with HIV transcriptional regulation. The approach of marking of HIV infected cells, combined with a single cell transcriptional analysis of HIV infection underscores the transcriptomic diversity within HIV reservoir and reveals gene regulation pathways potentially associated with HIV persistence.

303 SINGLE-CELL RNAflow-FISH REVEALS TRANSCRIPTIONAL DIVERSITY FOLLOWING LATENCY REVERSAL

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Background: “Shock and kill” cure strategies rely on the efficient induction of HIV transcription in latently infected cells by latency reversing agents (LRAs). Since latently infected cells are highly heterogeneous in vivo, investigating the activity of latency reversal agents at the single-cell level in clinical samples is crucial.

Methods: We developed a novel single cell multiplexed RNA flow cytometric assay for co-detection of viral RNA (vRNA: gagRNA, nefRNA and HIV exons) and p24 protein. We applied this approach to PBMCs from 10 ART-suppressed people living with HIV. We examined HIV transcription and translation induced by PMA/ionomycin, HDAC inhibitors (panobinostat, vorinostat; HDACi), and PKC agonists (bryostatin, PEP005; PKCa).

Results: We detected a median of 115 vRNA+ cells/10^6 CD4+ cells upon PMA/ionomycin stimulation, a frequency only 6.8-fold lower than integrated HIV DNA (median 783). Only a small fraction of these cells produced the HIV protein p24 (median 3.9%), which all co-expressed gag and nef RNA. Reactivated cells were dominated by transcriptionally heterogeneous vRNA+ p24+ cells: gag+ nef+ (median 47%) > gag+ nef+ (median 10%) > gag+ nef+ (median 12%). PEP005 and panobinostat resulted in robust latency reversal (median of 54 and 55 vRNA+ cells/10^6 CD4+ T cells, respectively). While PEP005 recapitulated the profile obtained with PMA/ionomycin, panobinostat induced an homogeneous gag + nef+ p24- population (median of 88% of vRNA+) with few nef RNA. These profiles were consistent within LRA classes. Combining PEP005 and panobinostat boosted reactivation at a level surpassing PMA/ionomycin (median 240 vRNA+ cells/10^6 CD4+ T cells with a profile similar to panobinostat, without p24 expression. We performed single-cell nestcd near full-length PCR on index sorted PMA/ionomycin-stimulated virus reservoirs to relate viral integrity to virus transcription. Highly deleted proviruses were enriched in gag + nef + p24- and gag + nef + p24+ populations. Both gag + nef + p24- and p24+ populations harbored potentially intact proviruses.

Conclusion: We identified distinct single-cell patterns of viral reactivation upon stimulation by latency reversal agents in clinical samples. Dissociated expression of structural and regulatory genes was frequent, and differences between LRA classes were observed. Regardless of the agent tested, only a minority of the cells in which HIV transcription was efficiently induced produced detectable levels of the HIV protein p24.
RESTING AND ACTIVATED CD4+ T CELLS BOTH HAVE SILENT AND ACTIVE HIV PROVIRUSES IN VIVO

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Background: Resting CD4+ T cells are thought to harbor transcriptionally-silent proviruses. We previously reported that only a small fraction of the cells in infected cell clones that persist on ART have proviruses expressing unspliced (us) HIV RNA (median 3%, range <1-65%). We evaluated whether these transcriptionally active HIV proviruses are preferentially found in activated rather than resting CD4+ T cells.

Methods: Leukopaks were obtained from donors in the ACTG A5341s study, the SCOPE cohort, and NIH trial protocols 57-1-0082 and 08-1-0221. Donors had been on effective ART for a median of 5 years (range 6.7-19.0 years). PBMC were FACs sorted on HLA-DR expression and collected in aliquots near an endpoint for infected cells. The number of infected cells was estimated before sorting from measurements of HIV DNA in total PBMC and from the percent CD4+ T cells. The samples were tested using our cell-associated RNA and DNA single-genome sequencing (CARD-SGS) assay to determine the number of infected cells, the fraction with us-HIV RNA, and the levels and sequences of HIV RNA and DNA in single cells.

Results: We assayed levels of us-HIV RNA in a median of 146,000 single HLA-DR+ and 1,052,500 single HLA-DR-CD4+ T cells from 6 participants. The frequencies of HIV-infected DR- and DR+ T cells were not different (median 0.1% in each subset). A median of 6% (range 4-9%) of the DR- cells and 4% (range 2-5%) of the DR+ cells expressed us-HIV RNA at the time of sample collection. Levels of us-HIV RNA in single DR- and DR+ cells were low (median 1.3 vs. 1.4 RNA copies/cell respectively). The HIV DNA sequences in the two subsets did not show differences in genetic diversity (average pairwise difference 1.9% vs. 1.7%) and were not genetically compartmentalized across the subsets (panmixia=0.6). In a cell clone with a replication-competent provirus that consisted of both DR- and DR+ cells, the fraction of cells with us-HIV RNA was 7% in both subsets.

Conclusion: Our finding that transcriptionally active proviruses are present at a similar frequency in HLA-DR- and HLA-DR+ T cells in people with HIV on ART supports the idea that the latent reservoir is not only in “resting” cells. The very low levels of HIV RNA in both HLA-DR- and HLA-DR+ T cell subsets also implies that cellular activation marker expression is not a reliable indicator of proviral activation in vivo.

COMPLETED GENOME-INTACT UNSPLICED HIV TRANSCRIPTS ARE RARE IN EX VIVO CD4+ CELLS

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Background: During virologic rebound, HIV-infected cells must produce 5' to 3' unspliced HIV transcripts. Conventional methods to study HIV transcription are unable to identify new virions. We have been on effective ART for a median of 9 years (range 6.7-19.0 years). PBMC were FACs sorted on HLA-DR expression and collected in aliquots near an endpoint for infected cells. The number of infected cells was estimated before sorting from measurements of HIV RNA in total PBMC and from the percent CD4+ T cells. The samples were tested using our cell-associated RNA and DNA single-genome sequencing (CARD-SGS) assay to determine the number of infected cells, the fraction with us-HIV RNA, and the levels and sequences of HIV RNA and DNA in single cells.

Results: We assayed levels of us-HIV RNA in a median of 146,000 single HLA-DR+ and 1,052,500 single HLA-DR-CD4+ T cells from 6 participants. The frequencies of HIV-infected DR- and DR+ T cells were not different (median 0.1% in each subset). A median of 6% (range 4-9%) of the DR- cells and 4% (range 2-5%) of the DR+ cells expressed us-HIV RNA at the time of sample collection. Levels of us-HIV RNA in single DR- and DR+ cells were low (median 1.3 vs. 1.4 RNA copies/cell respectively). The HIV DNA sequences in the two subsets did not show differences in genetic diversity (average pairwise difference 1.9% vs. 1.7%) and were not genetically compartmentalized across the subsets (panmixia=0.6). In a cell clone with a replication-competent provirus that consisted of both DR- and DR+ cells, the fraction of cells with us-HIV RNA was 7% in both subsets.

Conclusion: Our finding that transcriptionally active proviruses are present at a similar frequency in HLA-DR- and HLA-DR+ T cells in people with HIV on ART supports the idea that the latent reservoir is not only in “resting” cells. The very low levels of HIV RNA in both HLA-DR- and HLA-DR+ T cell subsets also implies that cellular activation marker expression is not a reliable indicator of proviral activation in vivo.

EXPRESSION OF CD32 IN HIV-RESERVOIR CELLS CONFRÉS RESISTANCE TO NATURAL KILLER CELLS

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Background: HIV establishes a persistent infection in cell reservoirs which are not susceptible to current antiretroviral therapy (ART). The expression of the Fcy receptor CD32 in infected cells has been identified as a marker of the active cell reservoir that persists during ART, but the mechanism by which these cells are maintained and evade the action of the immune system is currently unknown.

Methods: Antibody-dependent cell cytotoxicity (ADCC) and natural cytotoxicity (NC) NK functional responses were evaluated by flow cytometry in different subpopulations of CD4+ T cells after peptide-loading (n=30), ex-vivo infection (n=14) and viral reactivation from latency (n=6). Protein-defective viruses were used to elucidate the contribution of HIV to CD32 upregulation. Binding of HIV-specific antibodies, cell proliferation in response to immune complexes (IC) and expression of NK ligands HLA-E, MICA/B, CD155 and ULBP after ADCC and NC were measured by flow cytometry. The viral reservoir was assessed by quantification of total HIV-DNA and cHIV-RNA by qPCR in CD4+ T cells from ART-suppressed patients. Statistical comparisons were performed using Wilcoxon matched-pairs signed rank test, Mann-Whitney test, ANOVA Friedman test, and Spearman correlations, when appropriate.

Results: CD4+ T cells expressing CD32 were highly resistant to ADCC after peptide-loading (ANOVA p=0.001) (Figure 1), and ex-vivo infection (median % 19.6 vs. 35.8 for TCD32+ and TCD32-, p<0.01). This observation was particularly significant in ART-suppressed patients compared with elite controllers and non-suppressed donors.
healthy donors (median % 0.0, 34.8 and 45.0, for ART, EC and HD, p<0.01). Upregulation of CD32 was facilitated by the viral protein Nef (p<0.0001), decreased HIV-specific antibody binding (p<0.05), and conferred cell proliferation potential upon IC engagement (mean %K67 9.8 vs. 20.5 for basal vs. plasma HIV+, p<0.01). NK-resistant CD32-expressing cells expressed higher levels of HLA-E (median % 22.2 and 11.0, for TCDD2+ and TCDD3-, p<0.0001), but the administration of anti-HLA-E blocking antibodies, IFN-α or IL-15 did not reverse immune resistance. Furthermore, latently HIV-infected cells expressing CD32 upon viral reactivation resisted NK-killing (p<0.05) and an inverse correlation was observed between ADCC-killing and total HIV-DNA reservoir size (p=0.02).

Conclusion: We report a novel mechanism of viral evasion to NK-immune responses through the upregulation of CD32, which might represent a new obstacle to fully eliminate HIV.

Figure 1

Figure 1. Susceptibility of different cell subpopulations that comprise the HIV-reservoir to NK-mediated killing by ADCC in samples from ART-suppressed patients. The intrinsic cell susceptibility to ADCC was measured in naive [T0], stem cell memory [T0], central memory [T0], effector memory [T0], [T1], and TCM subsets. Statistical comparisons were performed using Wilcoxon Friedman test. Median with interquartile range is shown.

307 PROFILING THE PROVIRAL LANDSCAPE IN TISSUES FROM ART-TREATED INDIVIDUALS

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Background: HIV reservoir cells that circulate in blood have been well characterized, but little is known about the dissemination of HIV-1-infected cells across multiple anatomical tissues, especially the central nervous system (CNS). Here, we performed single-genome near-full-length proviral sequencing to evaluate the proviral landscape in multiple tissues from 2 ART-treated patients.

Methods: Frozen tissues derived from 2 ART-treated individuals, were sampled during autopsy. Genomic DNA was extracted, and HIV-1 DNA levels were initially quantified using the intact proviral DNA assay (IPDA), followed by single-genome near-full-length proviral sequencing.

Results: In patient 1, 577 HIV-1 sequences were detected in 14 different tissues from 754.77 million cells. Most of these proviruses were defective, and only a small fraction (4.5%, n=26) were intact, located in lymph node (n=18), spleen (n=3), colon (n=2), and kidney (n=3). The number of HIV copies varied from 0.02 to 6.24/million cells. The frequency of HIV sequences was highest in occipital cortex, followed by frontal cortex, basal ganglia and thalamus. Ten large clones of defective proviruses were also observed in lymph node, spleen, and kidney. Ten large clones of defective proviruses were also noticed, which were broadly distributed across lymph node, spleen, the CNS, the genitourinary system, and the gastrointestinal system. In patient 2, 5 different CNS sections were analyzed, with isolation of a total of 36 HIV sequences from 162.85 million cells. Only 1 intact sequence was detected in the basal ganglia. The number of HIV copies varied from 0.10 to 0.47/million cells. Basal ganglia had the highest frequencies of HIV sequences, relative to frontal cortex, ventricle, occipital cortex and thalamus. Three clones of defective proviruses were observed across multiple CNS sections.

Conclusion: Lymph node represents a tissue hotspot for viral persistence, while the CNS does not seem to be a major site for persistence of intact proviruses. Multi-compartment dissemination of clonal intact and defective proviruses occurred across multiple anatomical tissues, arguing against compartmentalization of HIV reservoir cells in specific tissue types.

308 HIV-INFECTED LYMPH NODE MIGRATORY DENDRITIC CELLS PERSIST IN ART-TREATED INDIVIDUALS

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Background: While pioneering studies demonstrated that HIV replication and spreading mainly occur in lymphoid tissues, the identification of specific cell subsets harboring replication competent virus in lymphoid tissues has long been neglected. In this context, we and others have recently shown that gut memory CD4 T cells, lymph node (LN) T follicular helper cells and tissue macrophages represent major HIV/SIV tissue reservoirs. LN dendritic cells (DCs) are endowed with an exceptional T-cell stimulatory potential and can either migrate from the periphery to the draining lymph node (nodal DCs) or locate in the LN for their entire life span (resident DCs). On the basis of these unique properties, long-term persistence of LN DCs infected with replication competent virus may represent the initial trigger of viral rebound post ART interruption and may therefore represent a major obstacle to HIV cure.

Methods: We therefore comprehensively assessed major virological parameters associated with HIV persistence in ex vivo isolated LN migratory and resident DCs isolated from viremic (N=3) and aviremic ART-treated HIV-infected subjects (N=7).

Results: LN migratory DCs harbored a much higher propensity for HIV infection in vitro than resident DCs (P<0.05) and supported HIV production, which was associated with significantly lower levels of SAMHD1 transcripts (P<0.05).

Interestingly, LN migratory DCs isolated directly ex vivo from viremic individuals harbored higher frequencies of cells harboring integrated HIV DNA, unspliced gag and multi-spliced tat-rev HIV RNA as compared to resident DCs. In addition, LN migratory DCs supported higher reactivation of HIV production (in the absence of CD4) and replication (in the presence of CD4) in vitro as compared to resident DCs. Interestingly, HIV-infected LN migratory DCs were still detectable in treated HIV-infected individuals and were able to support much higher levels of HIV production and replication when co-cultured with target CD4 T cells in vitro as compared to LN resident DCs (P<0.05).

Conclusion: The present study underscored a yet underestimated role of LN DCs in HIV persistence and may therefore highlight the need to adapt the currently explored experimental strategies aiming at purging viral reservoirs.

309 LONGITUDINAL DYNAMICS OF INTACT PROVIRAL HIV-1 DNA IN POSTTREATMENT CONTROLLERS

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Background: In HIV-1 infected individuals, discontinuation of antiretroviral combination therapy (cART) typically results in rapid viral rebound. However, a small number of individuals, termed post-treatment controllers (PTCs), exhibit sustained virologic suppression for months or years following cART interruption. The dynamics and evolution of the proviral reservoirs of these individuals are largely unknown.

Methods: Samples from 3 PTCs, who maintained undetectable or low viremia for up to 18 years after cART cessation, were longitudinally collected. Genomic DNA was diluted to single proviral genomes, followed by full-length individual proviral sequencing (FLIP-Seq) or matched integration site and proviral sequencing (MIP-Seq). Near full-length sequencing of single-genome HIV-1 plasma RNA was also performed.

Results: In total, 2633 individual proviral genomes were obtained. 59 integration sites of intact proviruses were identified, of which 47 were located at unique chromosomal positions. At baseline prior to ART interruption, the relative frequencies of total and intact proviruses in PTCs were comparable to a
background population of ART-treated individuals, with 23% (N=14) of intact proviruses being part of expanded clones. Notably, these clonally-expanded intact proviruses were frequently located in non-genic, centromeric satellite DNA (N=11, 79%); in contrast, non-clonal intact proviruses at baseline were preferentially located in genic chromosomal positions (N=22, 73%). Intact proviruses in expanded clones located in centromeric satellite DNA were repeatedly detected at multiple follow-up time points up to 14 years apart, while non-clonal proviruses integrated in genic chromosomal positions were selectively eliminated over time. 4–14 years after treatment interruption, 72-100% of all intact proviral sequences were clonally expanded and integrated in non-genic satellite DNA. In one PTC with plasma viral blips, we were able to obtain 12 near full-length plasma viral sequences, which showed close phylogenetic associations to non-clonal intact proviral sequences.

Conclusion: PTC display a unique integration site landscape with enrichment of intact proviruses in centromeric satellite DNA associated with deep latency, as previously shown for elite controllers. These data suggest that EC and PTC share similar underlying immune selection mechanisms that preferentially eliminate intact proviruses in chromosomal regions susceptible to reactivation signals, while intact proviruses in deep latency persist.

310 CELL-FREE DNA PREDICTS HIV REBOUND TIMING FOLLOWING ANTIRETROVIRAL THERAPY CESSION

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Background: The development of HIV cure strategies depends on our capacity to predict HIV rebound when antiretroviral therapy (ART) is stopped. Here, we applied a systems profiling approach to the analytical treatment interruption (ATI) framework, to identify circulating plasma factors that enable non-invasive prediction of viral rebound kinetics post-ART cessation.

Methods: Plasma samples were retrospectively collected from three Danish ATI cohorts (CLEAR, TEACH, REDUC, N=34 participants). The following assays were applied to pre-ATI (baseline) samples: 1) Cell-free DNA (cfDNA) abundance and fragment size were characterized using flow cytometry (Liq blot) and capillary electrophoresis (Agilent BioAnalyzer). 2) Extracellular vesicle (EV) abundance and composition were analyzed using nanoparticle tracking analysis (Malvern NanoSight) and flow cytometry-based measurement of 25 surface protein markers (BD LSR II). 3) The titer and antigen specificities of anti-HIV antibodies were measured by recombinant antigen binding (Ortho Vitros) and luciferase immunoprecipitation systems (LIPS) assays, respectively. 4) Circulating levels of 28 cytokines were measured by immunoassay (Luminex). Pearson correlations between biomarker levels and time to HIV rebound (days until viral load >50 copies/mL) and false discovery rate corrections were calculated using scipy and statsmodels Python libraries in custom Python scripts.

Results: Five features exhibited significant correlations with viral rebound timing after FDR correction. cfDNA abundance exhibited the strongest (positive) correlation with time until rebound (FDR<0.03; R2=0.62). The four remaining predictive features were EV surface proteins; frequencies of EVs expressing the eot-5'-nucleotidase CD73, glial fibrillary acidic protein (GFAP), fractalkine receptor CX3CR1, and major histocompatibility complex (MHC) class II were positively correlated with time until rebound (FDR<0.05).

Conclusion: Our systems approach revealed that elevated circulating cfDNA abundance and elevated expression of select immunomodulatory proteins on the EV surface are associated with delayed HIV rebound following ART cessation. These biomarkers can be conveniently and cost-effectively measured using small volumes of plasma, reinforcing their prognostic value. Our findings warrant confirmation in additional ATI cohorts. In addition, deciphering the mechanisms linking these predictive biomarkers to viral reactivation and immune control may lead to novel HIV cure approaches.

311 IMMUNE MARKERS AND TO TIME TO REBOUND DURING HIV TREATMENT INTERRUPTION IN ACTG A5345

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Background: Understanding factors that affect viral rebound timing during antiretroviral treatment interruption will accelerate efforts toward inducing sustained HIV remission. We evaluated whether immunologic parameters prior to treatment interruption are predictive of time to rebound in individuals interrupting ART in a highly monitored setting.

Methods: A5345 enrolled individuals who started ART in chronic or early infection and who were virally suppressed on ART for ≥2 yrs. Using flow cytometry, we evaluated frequencies of T cell maturation subsets, levels of T cell activation (HLA-DR+CD38+), exhaustion (PD-1, TIM3, TIGIT, LAG3, CD160), and HIV-specific T cell polyfunctional responses (CD107a, TNFa, IL2, IFNg) to gag, pol, and env peptide pools. Principal component analysis (PCA) and Spearman correlation were used to evaluate time to rebound ≥1000 cp/mL, and parameters were compared between participants rebounding ≤3wks vs. ≥4wks (Wilcoxon).

Results: Of the 45 analyzed participants, 33 were treated in chronic infection. There were no consistent differences in the immune parameters between early and chronic-treated participants. Higher frequencies of T cells expressing activation and exhaustion markers via PCA were modestly associated with shorter time to viral rebound (r = 0.27, p = 0.07) whereas none of the HIV-specific immune parameters correlated with viral rebound. 29 and 16 participants had viral rebound ≤3wks and ≥4wks, respectively. The two groups did not differ in terms of levels of T cell activation and exhaustion, except for a trend for lower %LAG3+ CD8+ T cells in the ≥4wk group (p = 0.06). The ≥4wk group also had greater %effectector memory CD4+ T cells (0.021) but lower %naive CD4+ T cells (p = 0.037). The ≥4wk group had lower absolute numbers of total CD8+ T cells expressing CD107A following HIV peptide pool stimulation (p = 0.05), and trends for lower %polyfunctional CD4+ T cell responses to pol (p = 0.07), env (p = 0.06) and total HIV peptide pool (p = 0.052).

Conclusion: Although no single immune marker was strongly predictive of time to rebound, higher levels of T cell activation and exhaustion while on ART are modestly associated with shorter time to rebound. In addition, those with early viral rebound had higher levels of on-ART HIV-specific T cells. Additional studies are needed to evaluate whether higher levels of proviral expression and antigenic stimulation on ART may be responsible for immune stimulation, exhaustion and more rapid viral rebound after treatment interruption.

312 MATHEMATICAL MODELING OF PREDICTORS OF POSTTREATMENT CONTROL IN HIV CURE TRIALS

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Background: Achieving an HIV cure or durable antiretroviral therapy (ART)-free HIV control is a significant unmet need. Due to the lack of validated predictors of virologic control, clinical trials rely on analytical treatment interruptions (ATI) to assess the efficacy of potential curative interventions. After ART is interrupted, a period of acute viremia occurs before the immune system responds, and a setpoint established. As this period of acute viremia poses risks to the participant and their sexual partners, algorithms that predict who will eventually control their virus might be helpful. Detailed studies of early viral dynamics in post-treatment controllers versus non-controllers may also provide insights into the development of an optimal therapeutic strategy.

Methods: We analyzed data from five AIDS Clinical Trials Group (ACTG) ATI studies (A371, A5024, A5068, A181, A197) and from the GS-US-382-3961 TLR7 study, in which prior controllers on ART interrupted therapy (N=134).

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Mathematical modeling and machine learning were used to identify early predictors of control at setpoint (defined by viral loads ≤400 copies/mL at 2/3 of timepoints for ≥24 weeks). Our analysis replicated real-time data collection in a clinical study, delineating outcomes for individuals who will become virologic controllers (N=20) from non-controllers.

**Results:** Our mathematical model identified the peak viral load, the rate of viral rebound (Slope-1), the time-to-peak and the time-to-rebound as the best predictors of virologic control following treatment interruption (Figure). These parameters identified individuals that became virologic controllers with accuracy, specificity and specificity scores (of ≥94%) (Peak+Slope-1+Time-to-rebound), (ii) ≥89% (Peak+Time-to-rebound), and (iii) ≥78% (Peak). Statistical analysis showed that viral peak is the most important predictor (p<0.0001), then Time-to-rebound (p=0.0019) and Slope-1 (p=0.026).

**Conclusion:** Early identification of virologic controllers is important to improve the safety and efficiency of ATI trials. During the immediate post-art period, peak viremia, the initial viral load slope and time-to-rebound predicted long-term post-treatment control. The quality of the host-response during the earliest stages of virus rebound may have long-term implications, suggesting that remission strategies will need to be optimized so that they are effective at the time the virus begins to spread systematically.

![Figure](image-url) Mathematical modeling identifies early predictors of virologic control.

The single-genome sequencing and full-length individual proviral DNA analysis showed that secondary syphilis. VL remained BDL until W76 post-ATI becoming detectable at W79 and W81 (both log10 =3.8 cp/mL on Nov 9th and 23rd 2020). Emerging HIV strain has the Brazilian GWGR motif at the tip of the V3 loop of gp120, whereas baseline strain presented the GPGR motif and had an incomplete N-linked glycosylation site suggesting a strain subjected to evolution and immune escape. The gag sequence from the emerging strain had amino acid substitutions compared to the original strain, but not in motifs corresponding to epitopes towards which cell-mediated immunity was directed. C2-V3-C3 sequences from baseline and after viremia are 17.3% different, whereas gag sequences are 11.4% different (Figure).

**Conclusion:** Anti-Gag cell-mediated immunity was associated with unprecedented post-therapy VL control in the chronic phase of the disease. Analyses are ongoing to investigate if the viral rebound source is the reactivation of a mutated virus or a new infection.

**Figure:** Amino acid alignment of C2-V3-C3 regions of gp120 (top) and gag (bottom) from baseline sample and sample collected upon viremia at 81 weeks post-ATI. Note: indicates similarities, and X the presence of more than one amino acid at the same position. The GPGR motif at the tip of the V3 loop is marked in yellow, and N-linked glycosylation site after the first cysteine is marked in blue. Sequences were obtained by bulk PCR from proviral DNA, and sequences from C2-V3-C3 regions obtained at weeks 40 and 44 during the intervention phase of the study are identical to the baseline sequence.

### 314 A NOVEL EXTENDED-LENGTH HIV-1 PLASMA RNA SEQUENCING ASSAY

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**Background:** The genetic characterization of full-length plasma-derived HIV-1 RNA is critical for identifying genetically intact genomes, as well as identifying genomes that are similar to those sequenced from proviral DNA. This remains technically challenging due to the instability of the RNA genome. We have developed an efficient procedure to sequence individual near full-length HIV-1 RNA genomes.

**Methods:** The single-genome sequencing and full-length individual proviral DNA sequencing assays were modified to allow for cDNA synthesis, PCR amplification at limiting dilution and sequencing of plasma-derived HIV-1 RNA genomes using primers spanning either GAG-3′ (8 kbp) or INT-3′ (5.1 kbp). Plasma samples from 2 untreated HIV-1-infected participants were diluted to copy numbers ranging from 10,000 to <50 copies to determine the lower limits and reproducibility of each region. To assess the extent of assay-related inter-template recombination, plasma samples containing 12000 HIV-1 RNA copies from each participant were mixed prior to RNA extraction and GAG-3′ sequencing. The error rate of the assay was calculated by analysing 95 GAG-3′ individual sequences (837520 total nucleotides) from PMA-activated J-Lat 10.3 culture supernatant.

**Results:** The lower limits of the GAG-3′ and INT-3′ assays were 350 copies and 40 copies, respectively. For the first participant (plasma collected approximately 1 month post-infection), 13.5% of GAG-3′ sequences were found to be genetically identical (n=140). This increased to 32.3% of INT-3′ sequences (n=93). For the second participant, whose plasma was collected during chronic infection, the GAG-3′ sequences were unique (n=110), while 1.96% of INT-3′ sequences were identical to another sequence (n=102). Using the GAG-3′ assay, 70% of sequences from the first participant were genetically intact, while 50% from the second participant were intact. Most defects were caused by a frameshift mutation found in Env. No examples of inter-template recombination were observed in the 87 single GAG-3′ genomes sequenced from the combined
participant plasma. The error rate of the GAG-3 assay was determined to be 0.0074%.

Conclusion: We have developed an assay to sequence near full—length regions of the HIV-1 RNA genome present in the plasma of HIV-1—infected individuals. This assay will provide a new level of sensitivity for understanding the genetic composition of plasma—derived HIV-1 RNA and assessing the genetic composition of rebound virus during an analytical treatment interruption.

315 QUANTIFICATION BIAS IN HIV-1 PROVIRUSES BY NEAR FULL-LENGTH GENOME SEQUENCING METHODS


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Background: Antiretroviral therapy (ART) completely inhibits HIV-1 replication but is not curative due to the establishment of a latent reservoir in resting CD4+ T cells, which remains the major barrier to cure. Proximal sequencing provides critical insights on how to measure the reservoir and distinguish the excess of defective proviruses from the intact proviruses which give rise to viral rebound. Near full length genome sequencing (nFGS) methods carried out at limiting dilution provide an estimate of the quantity of intact proviruses and a qualitative picture of the common fatal defects including hypermutation and internal deletions. However, nFGS methods assume that there is one to one correspondence between sequencing results and the actual frequency of proviruses. All nFGS methods rely on long distance PCR reactions, and interpretation of assay results are based on the assumption that all proviruses are amplified with equal efficiency regardless of length.

Methods: Here, we evaluate nFGS methods using the intact proviral DNA assay (IPDA) which quantitates intact and defective proviruses using short, highly efficient multiplex digital droplet PCRs. The IPDA can directly enumerate the number of product molecules generated by nFGS reactions. We measured the yield from nFGS reactions used in published reservoir assays on precisely quantitated templates with internal deletions of various lengths.

Results: We demonstrate that nFGS methods that employ long distance PCRs are extremely inefficient and underestimate full length (9kb) sequences by 70%. Deleted proviruses with shorter sequence length (3kb), representative of large internal deletions, were detected at a frequency of 96% and amplified with greater efficiency than full length proviruses.

Conclusion: These results demonstrate that reservoir assays that rely on nFGS do not give an accurate quantitative picture of the proviral landscape due to the inefficiency of long distance PCR. Accurate measurements of the latent reservoir of HIV-1 are critical in evaluating the efficacy of cure strategies. While nFGS methods provide detailed qualitative information, methods utilizing short highly efficient PCRs may provide a more accurate quantitative picture of the latent reservoir.

316 IMPROVED DETECTION OF HIV Gag p24 PROTEIN FROM PATIENT-DERIVED SAMPLES

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Background: Sensitive assays aimed at quantifying translationally competent genomes are needed to understand the contribution of viral proteins to HIV-1 pathogenesis and determine the effectiveness of cure interventions. Sensitive assays have been previously used to detect HIV gag p24 in blood and tissues, but these detection approaches still leave gaps with sensitivity and selectivity due to sample matrix effects. Here we report on an immunoprecipitation (IP) step in our p24 Simoa assay to overcome these barriers, leading to improved detection and expanded applications.

Methods: Conditions were optimized for p24 immunocapture and for conditions which efficiently elute p24 from the beads and maintain compatibility with the downstream assay. We applied the assay to ex vivo simulated blood CD4+ T cells and to rectal biopsies from both viremic and aviremic donors. Additionally, we developed new methodology for the extraction of protein from rectal biopsies, eliminating single cell isolations to enhance and simplify the protocol to ensure all sources of p24 are measured.

Results: IP of HIV gag p24 onto antibody-coated beads, followed by acidic elution and neutralization yielded nearly full recovery of all p24 in a sample. Validation of the approach was confirmed using recombinant p24 as well as patient—derived samples. Assay reproducibility was high and %CV was low as measured by inter—day experiments. Direct soaking of intact rectal pelvic biopsies in a lysis solution showed release of all CD4 protein from the biopsy (a surrogate marker for target cells of interest). The new assay shows high recovery and reproducibly even at low concentrations of analyte.

Conclusion: Including an IP step, HIV gag p24 detection by Simoa has been enhanced. This combined assay can detect as low as 1 fg of p24 protein from a given sample volume. The removal of matrix proteins prior to the read step, reduces background and false positives, aiding in data interpretation for low—level protein expression. The IP method allows for directly lysis of all cells in a rectal biopsy without having background from matrix effects. These steps reduce assay labor and minimize cell and protein during processing. These enhancements open additional avenues for ex vivo study of LRAs or clearance approaches using patient—derived samples.

317 ACCELERATED CEREBRAL BLOOD-FLOW REDUCTION AND BRAIN AGING IN PEOPLE LIVING WITH HIV

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Background: People living with HIV (PLWH) are characterized by altered brain structure and function. As they attain normal lifespans, it remains unclear whether HIV accelerates aging in select subgroups. Additionally, the relationship between viral load (VL) and brain aging has not been fully investigated.

Methods: Three groups were evaluated: HIV uninfected (HIV—) controls (n=206), PLWH with undetectable VL (<50 copies/mL; n=230), and PLWH with detectable VL (>50 copies/mL; n=93). A subset of individuals (n=201) completed longitudinal follow—up (mean=2.3 years post—baseline). T1—weighted structural imaging (TR/TE=2400/3.2ms) was used with a deep—learning algorithm to predict brain age based on a pre—trained model of healthy individuals. The gap between predicted and actual age (ΔAge) evaluated structural aging. Pseudo—continuous arterial spin labeling (TR/TE=3500/9.0ms, labeling=1500ms, post—labeling delay=1200ms) was obtained to calculate gray matter cerebral blood flow (CBF). Cognition was assessed with a 15—test battery that covered five domains. Mixed—effects linear models tested the prediction that detectable HIV VL was associated with accelerated aging as measured by greater reduction in CBF or increased structural ΔAge. Age, sex, and race were included as covariates. Relationships between cognition and CBF or ΔAge were explored.

Results: Age—associated CBF decline was not different between PLWH and HIV—controls. However, CBF reduction was accelerated in PLWH who had detectable HIV VL vs. undetectable HIV VL (p<0.02, A). In general, PLWH had accelerated structural ΔAge increases vs. HIV— controls (p<0.001, B), while structural aging did not differ between PLWH who had detectable and undetectable HIV VL. These effects represented significant age×group interactions. PLWH as a whole had reduced performance in executive function, psychomotor speed, and language, and those with detectable HIV VL had greater impairment in psychomotor speed and language (p’s<0.05). No association was observed between CBF and cognition. Across all participants, psychomotor slowing was associated with increased ΔAge (p<0.001).

Conclusion: Brain aging in PLWH included accelerated loss of gray matter perfusion and morphological alterations detected using machine learning. Cerebrovascular changes are sensitive to current HIV VL, while structural aging correlated with HIV serostatus but not HIV VL. Structural aging likely reflects cumulative gray and white matter degeneration, and is associated with cognitive function.


318 SEX DIFFERENCES IN WHITE-MATTER LOSS AND ASSOCIATED AXONAL DAMAGE IN PLWH

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Background: Studying sex differences in HIV-related co-morbidities has a global significance, as women now constitute the majority of adults aged 15 and over living with HIV worldwide. Specifically, higher levels of neurocognitive impairment have been reported in women living with HIV (WLWH) than men living with HIV (MLWH). This prompts the question of whether this difference is due to HIV-related brain changes in WLWH or better explained by other factors. Neuroimaging studies in people living with HIV (PLWH) have largely focused on comparing WLWH to women controls and MLWH to men controls or have studied one sex. By comparing brain volume proportion in virologically-controlled PLWH and controls of both sexes, we studied the effect of HIV and sex differences on brain health in the current era of widespread antiretroviral treatments.

Methods: Our prospective research cohort consists of virologically controlled PLWH and controls enrolled in a study of HIV and cognition. Volumetric measurements of participants’ MRIs were computed using Freesurfer software. Volumes were converted to proportions by dividing each by estimated total intracranial volume (eTIV), thus adjusting for skull size. To examine trends in volume loss over time, total brain, white matter, and gray matter proportions were plotted against age in each group to calculate slopes. Neurofilament light chain (NFL) in CSF was measured using the Quanterix Simoa platform.

Results: 286 scans from PLWH and 105 from age-matched controls were analyzed by Freesurfer. Brain volume proportions were overall higher in women compared to men (p < 0.001 Tukey’s multiple comparisons test) irrespective of HIV status. When looking at white and gray matter individually, results in Table 1 show significant differences between groups specifically in white matter slopes with evidence for the greatest loss of white matter over time in MLWH (p = 0.0151). A subset of participants (MLWH = 46, WLWH = 22) had CSF NFL measured concurrent with MRI. In this group, we found higher median NFL levels in MLWH compared to WLWH (p = 0.0151), suggesting axonal neurodegeneration following the same pattern as found in MRI sex differences.

Conclusion: In this well-characterized cohort of treated PLWH there are clear differences in brain volume proportions by sex with results suggesting MLWH may have more white matter loss over time that is associated with axonal damage.

Table 1: Comparisons of MRI Properties and Slopes

<table>
<thead>
<tr>
<th></th>
<th>MLWH (n=46)</th>
<th>Men- controls (n=22)</th>
<th>WLWH (n=22)</th>
<th>Women- controls (n=10)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Age Median (IQR)</td>
<td>53.3 (27)</td>
<td>54.07 (8.47)</td>
<td>54.67 (7.26)</td>
<td>52.85 (7.92)</td>
<td>0.4199</td>
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<tr>
<td>Brain proportion</td>
<td>0.70 (0.05)</td>
<td>0.70 (0.00)</td>
<td>0.70 (0.05)</td>
<td>0.74 (0.09)</td>
<td>&lt;0.001</td>
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<tr>
<td>White matter proportion</td>
<td>0.022621</td>
<td>-0.002533</td>
<td>0.01942</td>
<td>0.000341</td>
<td>0.8473</td>
</tr>
<tr>
<td>Gray matter proportion</td>
<td>0.30 (0.02)</td>
<td>0.30 (0.02)</td>
<td>0.32 (0.03)</td>
<td>0.32 (0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NFL slope</td>
<td>-0.0004875</td>
<td>-0.0004917</td>
<td>0.002665</td>
<td>0.0004896</td>
<td>0.0151</td>
</tr>
<tr>
<td>NFL cross-section slope*</td>
<td>0.010551</td>
<td>-0.001203</td>
<td>0.002213</td>
<td>-0.000777</td>
<td>0.7083</td>
</tr>
</tbody>
</table>

*slope = proportion over age in years

319 THE EFFECTS OF CARDIOVASCULAR HEALTH ON WHITE MATTER IN HIV+ AND HIV- PERSONS

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Background: Cardiovascular health has been linked to changes in brain structure and function in the HIV-negative (HIV-) population. However, results are still mixed as to how HIV and indicators of cardiovascular health may affect brain integrity, as measured by neuroimaging, in people living with HIV (PLWH).

Methods: 48 HIV- and 166 PLWH virologically well-controlled on stable combination antiretroviral therapy for >12 months, aged 30-80 years, completed cognitive testing and a magnetic resonance imaging scan, from which diffusion tensor imaging (DTI) values were computed to assess white matter microstructural integrity. Differences in Framingham Heart Study Cardiovascular Disease (10-year risk) scores (FRS) were evaluated between HIV- and PLWH. Multivariate general linear models assessed main effects and interactions between HIV serostatus and FRS categories (low risk=0-9, moderate risk=10-19, high risk= ≥20) on cognitive performance, and fractional anisotropy (FA) from 12 white matter tracts in the brain. Spearman’s correlations examined relationships between cognitive performance and tract FA.

Results: FRS was higher in PLWH (mean=17.8, SD=10.0) compared to HIV- (mean=11.1, SD=9.4) (p<0.001). Individuals in the moderate and high risk FRS groups performed significantly worse on tests of psychomotor speed compared to those in the low risk group (p-values < 0.01) regardless of HIV serostatus. There were no significant interactions between HIV and FRS (p-values > 0.05). There were no significant main effects of HIV serostatus on FA within the selected white matter tracts. However, regardless of HIV serostatus individuals in the moderate and high FRS groups demonstrated significantly lower FA of the frontal aslant tract (p-values < 0.01), frontal-occipital tract (p-values < 0.01), and the inferior longitudinal fasciculus (p-values < 0.01) compared to individuals in the low FRS group. There were no significant HIV and FRS interactions (p-values > 0.05) on FA measures. In general, lower FA was associated with poorer psychomotor speed performance (p-values < 0.01) regardless of HIV status.

Conclusion: PLWH demonstrate a significantly higher 10-year risk for cardiovascular disease compared to HIV- individuals, and that risk was associated with reduced cognitive performance and microstructural integrity of major white matter tracts in the brain. Cardiovascular health represents a potentially modifiable risk factor for reduced brain integrity in both HIV- and PLWH and should be treated in all individuals.
320 VASCULAR INJURY MARKERS ASSOCIATED WITH COGNITIVE IMPAIRMENT IN HIV PATIENTS ON ART
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Background: HIV-associated neurocognitive disorders (HAND) remain prevalent despite viral suppression on current antiretroviral therapy (ART). Cerebrovascular disease contributes to HAND, but biomarkers that distinguish vascular cognitive impairment from other types of HAND remain unclear. In this cross-sectional study, we investigated relationships between plasma and CSF vascular, inflammation, and CNS injury markers, HAND, and cerebrovascular disease in HIV+ subjects on ART.

Methods: Vascular injury (ICAM-1, VCAM-1, CRP), inflammation (IFN-γ, IL-1β, IL-6, IL-8, IL-15, IP-10, MCP-1, VEGF-A), and CNS injury (total Tau, GFAP, YKL-40) markers were measured in plasma and CSF samples collected from subjects enrolled in NNTC and CHARTER between 2006-2015 using the Meso Scale Discovery platform. Plasma samples were measured in 207 subjects (143 HIV+ virally suppressed on ART, age 30-75 years, 85% male, 71% white, 73 with HAND diagnoses of asymptomatic neurocognitive impairment (ANI) or mild neurocognitive disorder (MND) and 70 without HAND, and 64 HIV controls matched for age, gender, race). CSF and plasma albumin levels were measured and CSF/plasma albumin ratio (Qalb) was calculated.

Results: The median age of HIV+ participants was 52 years (IQR 47 – 58) and median CD4 count, CD4 nadir, viral load, and duration of HIV infection were 504 cells/ul, 76 cells/ul, 50 HIV copies/ml, and 16.5 years, respectively. HIV+ subjects had higher ICAM-1, CRP, IL-8, IL-15, IP-10, and VEGF in plasma and higher CRP, IP-10, VEGF, and GFAP in CSF compared with HIV- controls (<0.05). Plasma ICAM-1, VCAM-1, CRP, and YKL-40 and CNS injury markers (CSF total Tau, GFAP, YKL-40) were increased in HAND vs. no HAND or HIV- control groups and correlated negatively with neurocognitive T scores (<0.05). In contrast, most inflammation markers had weak or no significant associations with HAND and T scores. Cerebrovascular disease was more prevalent among HAND compared with no HAND subjects, and was associated with increased levels of VCAM1 and YKL-40 in plasma and increased total Tau and YKL-40 in CSF (<0.05). We did not detect significant associations between Qalb and plasma or CSF biomarkers.

Conclusion: Peripheral markers of vascular injury are more closely related to HAND and CNS injury in HIV patients on current ART than markers of inflammation, and may help to distinguish relative contributions of vascular cognitive impairment to HAND in this population.

321 SEX-SPECIFIC ASSOCIATIONS BETWEEN CSF MARKERS AND COGNITIVE FUNCTION IN PWH IN UGANDA
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Background: People with HIV (PWH) taking antiretroviral therapy (ART) have persistent cognitive impairment. The prevalence of cognitive impairment is higher in women with HIV compared to men with HIV, possibly due to sex differences in immune function. Here we report sex differences in cerebrospinal fluid (CSF) immune markers in relation to cognitive performance in the context of ART mediated viral suppression.

Methods: A subset of 83 PWH on ART (52% women; mean age=37.6 years) from the Rakai community cohort study conducted in rural Uganda completed a neuropsychological battery, depression and stress-related symptom questionnaires) or one year in conjunction with neuropsychiatric assessments (neuropsychological [NP] battery, depression and stress-related symptom questionnaires) or one year prior to the clinical assessments. Immune cell proportions were assessed in whole blood or freshly isolated PBMCs and flow cytometry. To examine associations between total monocytes, monocyte subsets, T-cell populations, and neurocognitive outcomes, we conducted Spearman’s Rho correlations (rs). Adjusted analyses were not required as none of our measured variables (age, CD4 count) met the definition of being a confounder.

Conclusion: Women with HIV have previously been shown to be more vulnerable to immune dysfunction and impaired cognition compared to men, including exhibiting higher CSF HIV viral loads. Here we find that men have a larger number of associations between CSF biomarkers and outcomes than women in the context of similar levels of CSF biomarkers of inflammation in men and women. Some of these patterns indicate a positive relationship between immune regulation and cognition, particularly in men. Our findings provide initial evidence that neuroinflammation may contribute to sex differences in cognition in PWH. Further investigation in larger cohorts and longitudinal studies may lead to delineation of sex-specific mechanisms of cognitive dysfunction in HIV and, possibly impact sex-specific screenings and management to limit the neurological complications of HIV in the ART era.
Results: A higher proportion of intermediate (CD14++CD16+) monocytes was associated with lower global NP function when assessing monocytes concurrently (rs=−0.60, P=0.006) and approximately one year before (predictive) NP testing (rs=−0.54, P=0.02). The same pattern was seen for mental flexibility (concurrent: rs=−0.53, P=0.01; predictive: rs=−0.68, P=0.003) and processing speed (concurrent: rs=−0.58, P=0.005; predictive: rs=−0.65, P=0.003). Conversely, there were no associations with monocyte subsets and mental health symptoms. However, lower CD4 T cell proportions were associated with higher perceived stress (rs=−0.38, P=0.03). A higher proportion of classical monocytes was also associated with better cognition (rs=0.48-0.67, P<0.05).

Conclusion: It is widely accepted that lentiviral infection of the CNS targets cells of monocyte-macrophage-microglial lineage and is associated with an increase in intermediate monocytes in the blood and monocyte migration into brain. However, the proportion of intermediate monocytes in blood of virally suppressed WWH has never been directly associated with cognition. Our findings provide initial evidence for a new, easily measured blood-based cognitive biomarker in WWH.

323 BASELINE MONOCYTE HIV RNA PREDICTS BLUNTED COGNITIVE TRAJECTORIES IN ACUTE INFECTION

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Background: Even when instituted during acute HIV infection (AHI), antiretroviral therapy (ART) does not completely prevent brain injury. Peripheral biomarkers of monocyte/macrophage activation are strong indicators of brain abnormalities in HIV. Whether early establishment of HIV in monocytes contributes to viral persistence and brain injury remains unknown. Here we determined the detectability of HIV RNA in monocytes and evaluated the levels of monocyte HIV RNA in acute HIV infection and its utility to predict long-term cognitive deficits following ART.

Methods: We isolated monocytes to ultra-high purity by flow cell sorting from cryopreserved peripheral blood mononuclear cells from 30 Thais who initiated ART during AHI (Fiebig stages I-IV). We isolated monocytes to ultra-high purity by flow cell sorting from cryopreserved peripheral blood mononuclear cells from 30 Thais who initiated ART during AHI (Fiebig stages I-IV). We isolated monocytes to ultra-high purity by flow cell sorting from cryopreserved peripheral blood mononuclear cells from 30 Thais who initiated ART during AHI (Fiebig stages I-IV). We isolated monocytes to ultra-high purity by flow cell sorting from cryopreserved peripheral blood mononuclear cells from 30 Thais who initiated ART during AHI (Fiebig stages I-IV). We isolated monocytes to ultra-high purity by flow cell sorting from cryopreserved peripheral blood mononuclear cells from 30 Thais who initiated ART during AHI (Fiebig stages I-IV).

Results: A total of 843 men were studied. 63% were HIV+, of whom 92% were on ART. In univariable analysis, increased levels of GlycA, CRP, and sCD14 (all p<0.01) as well as increased sCD163 (p<0.05) were associated with the presence of NPI. Hepatitis C (HCV) positivity was also associated with NPI. Among HIV+ participants, current detectable HIV RNA as well as cumulative HIV RNA over time (both p<0.01) were associated with NPI. In multivariable adjustment that adjusted for enrollment wave, BMI, estimated GFR (eGFR), and HCV status (Table), GlycA remained significantly associated with impairment. This was particularly true in HIV+ participants. Of models that incorporated the inflammatory biomarkers individually, CRP was the only biomarker significantly associated with NPI in addition to GlycA. When examining the association by tertile of CRP, GlycA was significantly associated with NPI only in the highest tertile of CRP.

Conclusion: Higher GlycA levels were independently associated with NPI in the MACS. GlycA therefore may be a durable window into NPI pathogenesis during HIV. Participants with higher CRP levels contributed significantly to this finding, suggesting that GlycA may be most valuable in the setting of increased overall inflammation, which is common during HIV.

<table>
<thead>
<tr>
<th>NPI</th>
<th>GlycA (log10)</th>
<th>CRP (log10)</th>
<th>sCD14 (log10)</th>
<th>sCD163 (log10)</th>
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<tr>
<td>Unvariable</td>
<td>1.47 (1.15, 1.79)</td>
<td>1.51 (1.02, 2.29)</td>
<td>1.38 (0.91, 1.90)</td>
<td>1.37 (0.91, 1.90)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.47 (1.20, 1.76)</td>
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<td>1.33 (0.83, 1.94)</td>
<td>1.33 (0.83, 1.94)</td>
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<tr>
<td>CRP adjusted</td>
<td>1.28 (1.01, 1.64)</td>
<td>1.53 (1.01, 1.98)</td>
<td>1.33 (0.83, 1.94)</td>
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<tr>
<td>sCD14 adjusted</td>
<td>1.13 (0.91, 1.34)</td>
<td>1.68 (1.24, 2.27)</td>
<td>0.80 (0.41, 1.34)</td>
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<td>sCD163 adjusted</td>
<td>1.20 (0.91, 1.49)</td>
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</tr>
<tr>
<td>Unvariable</td>
<td>1.79 (2.22, 2.60)</td>
<td>1.53 (0.83, 1.39)</td>
<td>1.47 (1.17, 1.89)</td>
<td>1.47 (1.17, 1.89)</td>
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<td>1.79 (2.22, 2.60)</td>
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<td>1.47 (1.17, 1.89)</td>
</tr>
</tbody>
</table>

324 GlycA IS ASSOCIATED WITH NEUROPSYCHOLOGICAL IMPAIRMENT IN PREDOMINANTLY HIV+ MEN

Albert M. Anderson1, Fiona Bhhoodoekham1, Dusica Curanovic2, Marge Connolly3, James Otos1, Wendy Post4, Erin D. Michos5, Cecile D. Lahiri1, Wendy Post6, Victor Valcour7, Carlo Sacdalan7, Eun Young Park2, Kamonkan Tangnaree5, Eric C. Seaberg7, James Becker1, for the MACS WHS Combined Cohort Study

1Emory University, Atlanta, GA, USA, 2Johns Hopkins University, Baltimore, MD, USA, 3LabCorp, Morrisville, NC, USA, 4The Johns Hopkins University, Baltimore, MD, USA, 5Northwestern University, Chicago, IL, USA, 6University of California Los Angeles, Los Angeles, CA, USA, 7University of Alabama at Birmingham, Birmingham, AL, USA, 8University of Pittsburgh, Pittsburgh, PA, USA

Background: Neuropsychological impairment (NPI) remains prevalent among people with HIV (PWH) in the combination antiretroviral therapy (ART) era. The mechanisms for NPI during ART remain unclear, but accumulating evidence suggests that chronic inflammation may have a role. GlycA, a novel biomarker representing the nuclear magnetic resonance signal from glycosylated acute phase reactants, is associated with coronary artery disease in PWH and may have additional promise as a marker of NPI.

Methods: We performed a cross-sectional analysis of HIV+ and HIV-negative men within the Multicenter AIDS Cohort Study (MACS), in which participants underwent regular comprehensive neuropsychological (NP) testing involving six domains. NP test scores are normalized based on age, sex, race, education, and previous test administration. NPI was present if two or more domains were at least one standard deviation below the mean, as per 2007 HIV Frascati criteria. In addition to GlycA, plasma concentrations of CRP, IL-6, IL-12, and sCD14 were measured. We examined associations between NPI and blood biomarkers using univariable and multivariable logistic regression incorporating medical covariates.

Results: A total of 843 men were studied. 63% were HIV+, of whom 92% were on ART. In univariable analysis, increased levels of GlycA, CRP, and sCD14 (all p<0.01) as well as increased sCD163 (p<0.05) were associated with the presence of NPI. Hepatitis C (HCV) positivity was also associated with NPI. Among HIV+ participants, current detectable HIV RNA as well as cumulative HIV RNA over time (both p<0.01) were associated with NPI. In multivariable adjustment that adjusted for enrollment wave, BMI, estimated GFR (eGFR), and HCV status (Table), GlycA remained significantly associated with impairment. This was particularly true in HIV+ participants. Of models that incorporated the inflammatory biomarkers individually, CRP was the only biomarker significantly associated with NPI in addition to GlycA. When examining the association by tertile of CRP, GlycA was significantly associated with NPI only in the highest tertile of CRP.

Conclusion: Higher GlycA levels were independently associated with NPI in the MACS. GlycA therefore may be a durable window into NPI pathogenesis during HIV. Participants with higher CRP levels contributed significantly to this finding, suggesting that GlycA may be most valuable in the setting of increased overall inflammation, which is common during HIV.
**Predicted Pathogenicity of mtDNA Variants and Motor Impairment in Persons with HIV**


**Background:** Mitochondrial DNA (mtDNA) variation is associated with neuropsychological impairment in persons with HIV. Prior studies have focused on mtDNA haplogroups. Recent models consider the cumulative impact of mtDNA variants predicted to be deleterious. MutPred software uses sequence conservation and protein structure to predict the impact of mtDNA variants on protein function. We examined MutPred pathogenicity scores in the CHARTER study, hypothesizing that persons with deleterious variants would be more likely to have NCI.

**Methods:** The CHARTER study included NC testing in persons with HIV from 2005-2008. MutPred pathogenicity scores were assigned to CHARTER participants with full mtDNA sequence; any single score >0.5 is considered potentially deleterious. Cross-sectional outcomes for cohort entry were NCI, defined by the global deficit score and seven NC domain deficit scores (DSSs), and by global and domain-specific mean T-scores (TS). Univariate comparisons used Wilcoxon rank sum and Fisher’s exact tests. Multivariable models were adjusted for age, sex, nadir CD4+ T-cell count, antiretroviral therapy, NC comorbidity status (incidental vs. contributing), and ancestry (European vs. non-European). Secondary analyses were ancestry-stratified.

**Results:** Data were available for 744 persons (357 African ancestry; 317 European). In univariate analysis of the overall cohort, the presence of any potentially deleterious variant was associated with motor impairment, with impaired persons less likely to have a deleterious variant (41 vs. 56%, p=0.001). In multivariable analysis, the presence of any deleterious variant remained associated with both motor impairment (p=0.03) and motor TS (p=0.05). In ancestry-stratified multivariable analyses, motor-domain impaired individuals of European and African ancestry were less likely to have a deleterious variant, although these results were not statistically significant (p=0.06-0.08). There was no significant association between motor TS and the presence of any deleterious variant in ancestry-stratified models.

**Conclusion:** Predicted pathogenicity of mtDNA variants was associated with motor performance in persons living with HIV, with impaired individuals significantly less likely to have any deleterious variant. These findings suggest that potentially deleterious variants may unexpectedly confer protection against impaired motor performance, perhaps through neuro-muscular pathways. Further studies are needed to explore the basis of these effects.

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**Buccal Mitochondrial DNA Is Associated with Amyloid-β 1-42 in Cerebrospinal Fluid**

Dipesh Solanky, Adam J. Fields, Ronald J. Ellis, Igor Grant, Robert K. Heaton, Scott Letendre, Sanjay R. Mehta

**Background:** Damage to mitochondrial (mt) genomes over time contributes to physiologic aging. Human immunodeficiency virus (HIV) infection is associated with premature aging, but measuring its impact is difficult due to a lack of reliable biomarkers. The mtDNA common deletion mutation (mtCDM) is a 4977-bp deletion associated with aging and neurodegenerative diseases. We examined how mtDNA copies per cell (cpc) and mtCDM cpc correlate with markers of neurodegeneration and inflammation.

**Methods:** Data from 149 adults were combined from two projects involving people with and without HIV (PWH and PWOH): 1) 78 PWH and 2) 46 PWH, 25 PWOH. We measured mtDNA and mtCDM cpc from buccal swabs by digital droplet PCR. Using univariable and stepwise multivariable regression, we compared them to disease and demographic characteristics and soluble biomarkers in cerebrospinal fluid (CSF) and blood measured by immunoassay.

**Results:** Median age of participants was 52 years, 81% men, and 53% white. Among PWH, 56% had antiretroviral therapy (ART, median 13.6 months); plasma HIV RNA was ≤200 cp/mL in 91%; and median CD4+ T-cell count was 595/µL. Median mtDNA level was 301 cpc (range 13.9 - 1432.2) and median mtCDM was 1.26 x 10⁴ cpc (0 - 14.7 x 10⁴) and both were higher in PWH (mtDNA: d=1.45, mtCDM: d=0.89, both p<0.0001). In the best model adjusting for HIV status and demographics, higher mtDNA cpc were associated with higher amyloid-β 1-42 (p=0.0005) and higher sTNR-II (p=0.042) in CSF (model R² = 0.37).

The association with amyloid-β 1-42 held in the subgroup of PWH, even after adjusting for duration of HIV (p=0.08) and ART (p=0.13), and nadir (p=0.53) and current (p=0.22) CD4+ count (model p<0.0001). Higher mtCDM cpc were associated with higher plasma sTNR-II levels (p=0.004) but no CSF biomarkers.

**Conclusion:** Buccal mtDNA is positively associated with amyloid-β 1-42 in CSF and both mt biomarkers were positively associated with sTNR-II. Increased mtDNA cpc may indicate greater amyloid-β and sTNR-II efflux from the brain into CSF. Further studies are needed to understand the concomitantly increased mtCDM, but this may reflect an increase in mt activity and oxidative stress due to HIV and ART effects in the brain. Our findings also support the use of affordable, easily accessible buccal specimens as a screening tool for inflammation and amyloid-β in CSF. Additional confirmatory and mechanistic studies on mt genome alteration by HIV and ART may identify interventions to prevent or treat neurodegenerative complications.
HIGHER COMORBIDITY BURDEN PREDICTS WORSENING NEUROCOGNITION IN PEOPLE WITH HIV
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1University of California San Diego, La Jolla, CA, USA, 2University of California San Diego, San Diego, CA, USA

Background: Comorbidities linked to aging such as diabetes mellitus, visceral adiposity and renal dysfunction accumulate at a faster rate in people with HIV (PWH) than in the general population. We evaluated whether the Charlson Index, a global comorbidity scale comprising 17 variables that has been validated in previous studies, predicted neurocognitive trajectories in PWH.

Methods: Neurocognition was measured by averaging scaled scores from all assessments in a comprehensive neuropsychological battery. Multilevel modeling was used to examine between- and within-person predictors of global neurocognition. At the between-person level, average Charlson Index (averaged within each person across all their visits) was examined as a predictor of neurocognitive change over time, covarying for the effect of HIV disease characteristics (proportion of visits virally suppressed, average CD4) At the within-person level, Charlson Index was used to predict fluctuations in global neurocognition at the same and next visit, covarying for visit-specific effects of viral load detectability and current CD4 count.

Results: Participants were 1195 PWH (mean age at baseline = 43.0; SD 9.7) followed for an average of over 7.1 years (SD = 5.0; range = 0.5 to 20.5 years). Between persons, higher average Charlson index scores were associated with faster rates of global neurocognitive decline (standardized β = -0.50 [0.015], p = 0.001). This global effect was driven by significant decline in the domains of executive functioning (β = 0.001) and working memory (p = 0.007). The Figure shows the contributions of individual components of the Charlson. HIV disease characteristics did not predict trajectories of neurocognitive change (p > 0.05). At the within-person level of the model, lower current CD4+ lymphocytes (β = 0.043 [0.009]; p < 0.001), detectable plasma HIV RNA (β = 0.018 [0.006]; p = 0.001), and higher Charlson Index score (β = -0.046 [0.015]; p = 0.003) related to worse concurrent global neurocognitive performance at the same visit. Time-lag analyses demonstrated that increasing comorbidities occurred concomitantly with, not before, neurocognitive decline.

Conclusion: The impact of comorbidities on trajectories of neurocognitive decline was greater than that of HIV disease factors. Although correlative, the temporal relationship between accumulating comorbidities and neurocognitive decline suggests that interventions to prevent or ameliorate a variety of comorbidities may improve neurocognitive prognosis for PWH.
people with HIV (PWH) on antiretroviral therapy (ART) and people without HIV (PWoH) in primary care. Methods: We conducted a cohort study of individuals aged ≥50 years who were members of Kaiser Permanente health plans in Northern California, Southern California, and Mid-Atlantic States (Maryland, Virginia, Washington D.C.) between 2000 and 2016. PWH and PWoH were frequency-matched 1:10 by age, sex, race/ethnicity, medical facility, and calendar year at baseline. We excluded PWH without an ART prescription fill in the year before baseline. Incident all-cause dementia diagnoses were identified in electronic health records using International Classification of Diseases codes confirmed via chart review in ~300 randomly selected patients. Demencia incidence was evaluated by HIV status using Poisson regression models adjusted for period (2000-2004, 2005-2009, 2010-2014, 2015-2016), age (50-59, 60-69, ≥70 years), sex, race/ethnicity (White, Black, Hispanic, Other), and healthcare utilization (outpatient visit frequency in the year before baseline). An overall model adjusted for all covariates. Subsequent adjusted models were stratified by period, age, sex, and race/ethnicity.

Results: The study included 11,302 PWH and 154,620 uninfected individuals (mean baseline age=53 years, 11% female; 82% of PWH with HIV RNA<200 copies/mL). During follow-up (mean=8 years), 2,64 PWH and 2,06 PWoH developed dementia. Crude incidence of dementia was 4.4 (95% CI=3.9-4.9) and 2.1 (95% CI=2.0-2.2) per 1,000 person-years in PWH and PWoH, respectively (incidence rate ratio [IRR]=2.0, 95% CI=1.8-2.3). After covariate adjustment, incidence remained higher in PWH (IRR=1.9, 95% CI=1.7-2.2). In models stratified by age, IRR was highest in younger age groups (age 50-59, IRR=3.3, 95% CI=2.7-3.9; age 60-69, IRR=1.7, 95% CI=1.3-2.1) but normalized in those age ≥70 (IRR=1.0, 95% CI=0.7-1.3). In models stratified by period, IRR decreased over time but remained elevated among PWH in the most recent (2015-2016) period (IRR=1.7, 95% CI=1.2-2.3). There were no significant differences in incidence across sex and race/ethnicity strata.

Conclusion: Despite ART use, dementia incidence is higher among PWH compared with PWoH and is diagnosed at younger ages. Further research is needed to determine factors contributing to age-specific patterns and continued elevated dementia incidence among ART-treated PWH.

![Graph showing incidence rates ratio of dementia over time for PWH and PWoH](image)

**Figure 1.** Global Mean T-Score Trajectories Over Time by aMCI/HAND Group among PWH 45+ years old; Diagnostic Category Determined at Baseline

### 332 EPIGENETIC AGING ASSOCIATED WITH COGNITIVE IMPAIRMENT IN OLDER BLACK ADULTS WITH HIV


*Rutgers School of Public Health, Piscataway, NJ, USA, *Columbia University Medical Center, New York, NY, USA*

**Background:** Several studies detected epigenetic age acceleration using a DNA methylation (DNAm)-based biomarker of aging in people with HIV (PWH), but data in African Americans (AA), women, and older PWH are lacking. We assessed if HIV infection is associated with epigenetic age acceleration in AA older adults, and evaluated if epigenetic age acceleration is associated with cognitive function.

**Methods:** We measured DNAm in whole blood using Illumina EPIC Arrays in 107 (69 HIV− and 38 HIV− controls) AA men and women ages 60-75 living in New York City. We estimated three age acceleration measures, where positive values indicate that the blood sample is older than expected based on chronological age: epigenetic age acceleration (EAA), extrinsic epigenetic age acceleration (EEAA), and intrinsic epigenetic age acceleration (IEAA). The NIH Toolbox Cognition Battery was used to assess cognitive function across five domains: executive function, attention, working memory, processing speed, and language. We compared age acceleration measures between groups using t-tests and assessed correlations between age acceleration measures and standardized cognitive function scores, by HIV group.

**Results:** The HIV− and HIV− groups did not differ by sex (49 vs 42% female), chronological age (65 vs 66 years), ethnicity (93% not Hispanic or Latino), frailty by Fried criteria (36 vs. 32%), or mean BMI (28.5 vs 30.9 kg/m²). 83% of the HIV+ had a viral load <50 copies/mL and 94% had a recent CD4 ≥200 cells/
Blood cell composition differed between groups, largely driven by higher proportions of CD8 (0.35 vs 0.18, p<0.01) and lower proportions of CD4 T-cells (0.23 vs 0.38, p<0.01) in the HIV+ group. Chronological age correlated with DNAm age (r=0.36, p<0.01). HIV+ had a higher mean EAA (2.4±8.5 vs -4.3±5.6, p<0.01) and EAA (1.4±10.4 vs -2.5±5.6, p<0.01) compared to HIV-. IEAA was not significantly different between groups (0.2±6.1 vs -0.4±5.0, NS).

There were negative linear relationships between EAA and IEAA and attention (r=-0.24, p=0.05), t=-0.26, p=0.03, respectively) and working memory (r=-0.26, p=0.03; t=-0.30, p=0.01) for the HIV group, but not the controls.

Conclusion: Epigenetic age acceleration in blood was observed in AA older PWH using two measures, including IEAA which reflects immunosenescence. There was no evidence of age acceleration independent of cell type composition (IEAA) associated with HIV, but this measure was associated with decreased cognitive function in the HIV group.

334 IMPACT OF SWITCH FROM EFV/F/TDF TO B/F/TAF ON PSYCHIATRIC SYMPTOMS AND NEUROCOGNITION

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1Lauroz Spallanzani National Institute for Infectious Diseases, Rome, Italy

Background: EFV has been associated with neuropsychiatric side effects and sleep disorders, while association with neurocognitive impairment (NCI) remains controversial. Our aim was to investigate whether a treatment switch from EFV/F/TDF to B/F/TAF may improve psychiatric symptoms, sleep function and if it has an impact on neurocognition.

Methods: EBONY is a pilot, single-arm, open label, prospective study of HIV suppressed patients (pts) on the efficacy and safety of switching from EFV/F/TDF to B/F/TAF. All pts underwent neuropsychological assessment (NPA) at the switch (T0) and after 48 weeks (T1). NPA was carried out through a standardized battery of 12 tests (5 domains). Participants were classified as having NCI if they scored >1 standard deviation (SD) below the normal mean in at least 2 tests, or >2 SD below in 1 test. Individual z-scores were determined, and NPZ-12 was calculated as the average of the 12 test z-scores; changes of NPZ-12 were analyzed as outcome. HAND was classified by Frascati’s criteria. Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI) and Pittsburgh Sleep Quality Index (PSQI) were administered. Paired Wilcoxon and McNemar tests were used for statistical comparisons. Multivariable linear regression was used to find factors associated with changes in tests.

Results: 109 participants: mostly Caucasian male with a median age of 53 years (IQR 46-58), 30% MSM, median education 13yrs, 8% with at least 1 comorbidity and median CD4 count of 604 cells/mm³, (500-810). The median time of EFV/F/TDF therapy was 8.4 yrs (7.1-10.1). NPA revealed a NCI in 36/109(33%) pts at T0 and 37/109(34%) at T1 (p=0.866). Specifically: 67.9% pts did not change at T1, 16.5% worsened and 16.6% improved. At T1 we observed a slight worsening in the NC function (mean±SD NPZ12 change: -0.21;+0.36; p<0.001;Figure 1a) and in mental flexibility (-0.16;+0.63; p=0.003), working memory (-0.47;+0.75; p<0.001) and memory (-0.28; +0.85; p<0.001) domains. We observed an ANI in 3.7% at T0 and 7.3% at T1. No pts with MND or HAD were found. Self-reported BAI and BDI-II questionnaires revealed an improvement at T1. No factor associated with test score changes was found.

Conclusion: Our results suggest that switch from EFV/F/TDF to B/F/TAF significantly improves psychiatric symptoms and sleep disorders. Neuropsychometric performance remained substantially stable, even though a decline on NPZ-12 and in specific domains was observed.
### 335 The Use of Less Neurotoxic Antiretrovirals: Secondary Endpoints of the MARAND-X Study

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**University of Turin, Turin, Italy, 1 ASL Città di Torino, Turin, Italy, 2 Istituto Oncologico di Toscana, Arezzo, Italy, 3 Istituto Superiore di Sanità, Rome, Italy**

**Background:** Despite a high prevalence (30-50%) HIV-associated neurocognitive disorders pathogenesis is incompletely understood. Antiretrovirals' neurotoxicity has been suggested as a potential mechanism. Aim of the study was to measure the change in resting EEG waves, CSF biomarkers and Fibroscan measurements in PLWH with HAND randomized to a less neurotoxic regimen (darunavir/cobicistat, maraviroc, emtricitabine "MARAND") or continuing their treatment.

**Methods:** Adult PLWH with HAND were enrolled if presenting no major resistance-associated mutations, not on efavirenz/darunavir, with R5-tropic HIV, without major confounding conditions, >6 months after HCV-SVR and with plasma and CSF HIV RNA <50 copies/mL. After 1:1 randomization, tests were repeated at 24 weeks: resting EEG waves, CSF biomarkers and Fibroscan measurements. Data are expressed as median (interquartile range). Non-parametric tests (Mann-Whitney and Wilcoxon's) were used.

**Results:** In June 2020 the study was prematurely terminated for slow accrual when 38 participants were enrolled (19 per arm). Male (76.3%) and European ancestry (92.1%) were prevalent. Median age was 55 years (51-60). Plasma and CSF HIV RNA were <20 copies/mL in 33 (86.8%) and 32 (84.2%) participants; median CD4+ count was 626 cell/µL (469-772). Baseline characteristics were similar between the study arms. LORETA delta and alpha waves were similar at baseline and W24 (n=29). A significant decrease in parietal delta waves was observed in the MARAND arm (-0.69, p=0.022) but not in other waves or cortical sources. CSF HIV-RNA (n=14) was detectable in 43-44% participants at baseline and W24 with no significant difference. A significant decrease in CSF p-tau (14.6 pg/mL, p=0.018) and an increase in CSF neopterin (+1.87 ng/mL, p=0.045) were observed in the MARAND arm. Fibroscan (n=25) stiffness and coefficient attenuation parameter (CAP) were similar at baseline and W24: we observed a significant reduction in stiffness at W24 in the MARAND arm (-0.8 KPa, p=0.038).

**Conclusion:** Conclusions: Despite a small sample size we observed improvement in EEG cortical sources and in hepatic stiffness in patients randomized to the experimental arm. CSF biomarkers changes (lower phosphorylated-tau and higher neopterin) need to be replicated in large cohorts.

### 336 Pembrolizumab for Progressive Multifocal Leuкоencephalopathy (PML) in PLWH

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**IAS–USA**

**Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy**

**Background:** PML is a severe demyelinating disease occurring in advanced HIV infection, caused by the reactivation of poliomavirus JC (JCV). In absence of specific anti-JCV therapy, immunity restoration induced by effective combined antiretroviral treatment (cART) is a possible treatment strategy. The rationale on Pembrolizumab (PEM) use for treatment of PML is the inhibition of programmed cell death protein 1 (PD-1) potentially improving anti-JCV-specific response with consequent JCV clearance.

**Methods:** We used PEM (2 mg/kg/iv every 4 wks) with cART for treatment of PML. The drug was given on a compassionate-use basis and all patients (pts) provided written informed consent. At each administration were performed clinical evaluation, MRI and laboratory testing, including immunophenotyping (CD3, CD4, CD8, PD-1 markers) in blood and CSF by flow cytometry. JCV-specific T-cells response was analysed by Elispot assay after stimulation with JCV peptides. HIV-1 RNA was quantified with the Amplisense HIV-1 Quant DX assay (Hologic) with a LoQ of 30 cps/ml. JCV DNA by an in house RealTime PCR method on LightCycler (Roche), targeting VP1, with LoD of 150 cps/ml.

**Results:** At present, 5 HIV+ pts enrolled: 4 male, median age 43 yrs (29-52), median CD4 and CD8 count 150 (15-158) and 973 (354-1250) cell/µm³, respectively; median JCV-DNA and HIV-RNA in CSF/plasma pairs 9,540/1,503 cps/ml and 2,230/619 cps/ml. All pts received at least two doses, with a maximum of seven doses (in pt1). After treatment, we observed a JCV-DNA decline in all pts (median change -0.42, -1.64, -0.09 log). 3/5 pts showed a stability of the clinical picture and neuroimaging (pt1, pt3 and pt4), and two others died (pt2 and pt5). PD-1 expression was high in circulating CD4 and CD8 at baseline, gradually decreased over time and remained stable at low level in all patients. Expression of PD-1 in CSF was higher than in the peripheral blood, even though lower after PEM. All pts experienced an improvement of JCV-specific T-cell response after PEM that paralleled PD-1 decrease and JCV-DNA decay both in CSF and in plasma; all pts still alive showed undetectable JCV-DNA values in both compartments.

**Conclusion:** According to these preliminary data, JCV-DNA quantitative reduction, PD-1 down regulation and enhanced JCV-specific T-cell response after pembrolizumab was observed, even though clinical and radiological response remained poor. More data are needed in order to identify predictors of response to therapy.
337 INTRANASAL INSULIN IMPROVES ATTENTION AND MEMORY IN PEOPLE WITH HIV

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2Background: Despite suppression of HIV replication with antiretroviral therapies (ART), cognitive impairment (CI) remain prevalent in virally suppressed people with HIV (VS-PWH). Although the precise mechanisms for these residual CI are not fully understood, there is considerable evidence that brain energy metabolism is progressively impaired in VS-PWH. We hypothesized that intranasal insulin (INI) would enhance brain energetics in VS-PWH with consequent improvements in cognition.

Methods: In a randomized, double-blind, placebo-controlled study, 21 non-diabetic VS-PWH with mild-to-moderate CI were randomized to receive INI (20 IU/day/nare) or placebo. Participants completed standardized neuropsychological (NP) tests at baseline, 12, and 24 weeks. Demographically adjusted Z-scores were created for each outcome using the best available norms. Primary outcomes included Global Deficit Score (GDS), NPZ-8, and performance on individual NP tests. A series of mixed-effects regressions were conducted to examine the change in cognitive performance over 24 weeks as a function of Treatment Group. Models were adjusted for depressive symptoms.

Results: Of the 45 candidates screened, 21 met criteria for the study; top reasons for exclusion were insufficient CI (n=10) and current illicit drug use (n=8). No serious study-related adverse events were reported. Six participants discontinued the study early due to naso-phyaryngeal irritation (n=3), non-compliance (n=2), and unrelated medical illness (n=1). There were no significant treatment group differences at baseline on any NP outcome (p>0.05 for all comparisons; Student’s t-test). A mixed effects regression of GDS over time with cross-product between INI and time demonstrated a significant treatment group effect (p=0.029) with improved GDS in the INI group at 12 and 24-weeks compared to placebo. Improvements on individual NP tests were apparent on measures of verbal memory (HVLT-R delayed free recall, p=0.028) between baseline and 24-weeks, on visual memory (Rey delayed recall, p=0.002) and attention (Trail Making Test-Part A, p=0.006) between baseline and 12-weeks in the INI group compared to placebo. There were no Treatment Group differences over time on the NPZ-8.

Conclusion: In this pilot study, INI improved performance on NP tests related to memory and attention in VS-PWH. These findings warrant further investigation of intranasal insulin as a cognitive enhancer in VS-PWH.

338 STATIN USE AND COGNITIVE PERFORMANCE IN THE MULTICENTER AIDS COHORT STUDY

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Background: Cardiovascular and cerebrovascular ischemic disease risk factors are associated with cognitive impairment in HIV seropositive individuals. Hypercholesterolemia, one such risk factor, is often treated with statin medications. Statin medications have both lipid lowering effect and anti-inflammatory properties, can improve endothelial function, and enhance dynamic blood flow in cerebral small vessels, which could potentially benefit cognitive function.

Methods: Using data from 1,407 participants in the Multicenter AIDS Cohort Study who either ever or never initiated statins, we explored the relationship between statin use and cognitive performance such that statin initiators tended to have, on average, a faster rate of cognitive test performance decline. HIV-serostatus did not significantly modify our results for the majority of cognitive tests that were explored.

Conclusion: After adjusting for confounders, cognitive test performance did not markedly differ between statin initiators and non-initiators at time proximal to initiation, but, longitudinally, statin initiators’ test performance declined more quickly. HIV-serostatus did not modify these results.

Results: After adjusting for potential confounders, statin initiation was not significantly associated with performance on any neuropsychological test either at the last test completion prior to statin initiation or for the first test completion after statin initiation. Longitudinally, statin use was strongly associated with cognitive test performance such that statin initiators tended to have, on average, a faster rate of cognitive test performance decline. HIV-serostatus did not significantly modify our results for the majority of cognitive tests that were explored.

339 REVERSIBILITY OF SLEEP DISTURBANCES AFTER SWITCHING FROM DTG/3TC/ABC TO DRV/C/FTC/TAF

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Background: Evidence supports switching DTG/3TC/ABC in patients complaining about insomnia. However, there is unknown if the benefit observed could also apply to non-complaining patients displaying sleep disturbances in self-reported questionnaires used as screening tools, such as the Pittsburgh sleep quality index (PSQI).

Methods: We designed the DETOX study, as an open label, randomized (1:1), multicenter, pilot clinical trial, to evaluate the reversibility of sleep disturbances detected with the PSQI in well-suppressed patients on DTG/3TC/ABC (>12 weeks) not complaining of insomnia. Participants with a PSQI >5 were randomized either to switch to DRV/c/FTC/TAF for 8 weeks (arm 1) or to continue 4 weeks on DTG/3TC/ABC and then switch to DRV/c/FTC/TAF for 8 weeks (arm 2). Every 4 weeks, participants self-reported using the PSQI, the Hospital Anxiety & Depression Scale (HADS) and a questionnaire exploring about 11 neuropsychiatric adverse events (AE). Raw scores on PSQI and HADS, along with an average score from adding the grade of each neuropsychiatric AE, were normalized (0-100). When we compared changes at week 4 (between study arms) and after participants completed 4 and 8 weeks on DRV/c/FTC/TAF, Additional analyses included virological outcomes.

Results: The study included 72 participants (arm 1: n=37; arm 2: n=35). Both arms had similar baseline characteristics. Three discontinued prematurely before week 4 (arm 1: none; arm 2: 1 COVID-19, 1 loss of follow up (LFU) and 1 consent withdrawal). At week 4, we observed significant improvements (arm 1 vs. arm 2) in PSQI (mean change±SD: 11.5±10.2 vs. 0.6±8.9; p=0.003) and HADS anxiety scale (14±16.9 vs. 1.9±15.6; p=0.003) and AE (13.7±13.3 vs. 1.3±8.6; p<0.001) scores. Sixty-nine participants switched to DRV/c/FTC/TAF: 37 at baseline (arm 1) and 32 at week 4 (arm 2). All except 3 who discontinued prematurely (2 LFU and 1 due to AE nausea) completed 8 weeks of follow up.
Pooled analysis showed significant improvements in most neuropsychiatric scores and symptoms (table), with no virologic failures reported. After switching to DRV/c/FTC/TAF, 26 participants (37.7%) reported any AE (all grade 1-2). Most common AE were headache (7.2%) and dyslipidemia (7.2%).

**Conclusion:** Sleep disturbances detected through self-reported screening tools seem to be associated with patients on DTG/3TC/ABC not complaining of insomnia. These disturbances, among other neuropsychiatric symptoms such as anxiety or depression, could improve after switching to DRV/c/FTC/TAF.

**TABLE** Changes in CNS-related scores and symptoms at week 4 and after switching from DTG/3TC/ABC to DRV/c/FTC/TAF

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4 after switching to DRV/c/FTC/TAF</th>
<th>Week 8 after switching to DRV/c/FTC/TAF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS-related adverse events, mean/IQR</td>
<td>26.9/2</td>
<td>13.7/1.5</td>
<td>9.3/1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hospital Anxiety and depression scale (Anxiety subscale), mean/IQR</td>
<td>36.8/2.3</td>
<td>26.1/2.1</td>
<td>22.2/1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hospital Anxiety and depression scale Depression subscale, mean/IQR</td>
<td>23.0/2.0</td>
<td>16.9/1.7</td>
<td>16.0/1.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Neurotic-somatic quality index, mean/IQR</td>
<td>36.8/2.3</td>
<td>27.6/1.6</td>
<td>23.5/1.5</td>
<td>&lt;0.001</td>
</tr>
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</table>

**Methods**: RV254 cohort participants initiated ART at a median=0[IQR 0–1] days after AHI diagnosis (Fiebig I-V) and completed the 9-item Patient Health Questionnaire (PHQ-9, score 0–27) for depression symptoms and the 11-item Patient’s Rating of Functional Impairment (PROFI, score 0–6) to assess depressive symptoms and self-assessment of function.

**Conclusion**: In those who maintain viral suppression after ART initiation during AHI, depression symptoms remain stably improved at 6 years. While overall, PHQ-9 score at 6 years correlates with baseline score during AHI, moderate-severe depression symptoms during AHI do not portend durable depression symptoms or impaired immunologic recovery after long-term ART.

**341 HIV REPLICATION IN THE CNS IS ASSOCIATED WITH NEUROCOGNITION AND DEPRESSION PRE-ART**

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**Background**: People with HIV (PWH) experience more neurocognitive impairment and depression than uninfected individuals, particularly in resource-limited settings. However, initiating ART often returns neurocognition and depression to levels observed for uninfected people. Here we test the hypothesis that neurocognition and depression are substantially impacted by HIV replication in the CNS, beyond the effects of systemic replication or stigma related stress.

**Methods**: We successfully sequenced viral populations in cerebrospinal fluid (CSF) and blood collected from 50 ART-naive PWH enrolled in the Rakai Neurology Cohort Study (RNCS; sequencing attempted for 169 PWH). Viral RNA was isolated from plasma and CSF and Illumina MiSeq deep sequencing with Primer ID was used to sequence env V1-V3. Identification of a CSF viral lineage that was genetically distinct from HIV in the blood (i.e. compartmentalized) was evidence of sustained viral replication in the CNS. The viral burden of HIV replication in the CNS was estimated as the Compartmentalized CSF Viral Load (CCVL = the percent of CSF-derived sequences that are CSF-specific vs. CSF viral load). All 50 individuals completed a neuropsychological test battery, assessment of depressive symptoms (CES-D) and self-assessment of function (PAOF).

**Results**: The mean CCVL in this cohort of 50 was 3.5 log10 RNA cp/ml (SD=1.72). Higher CCVL was associated with slower processing speed (p<0.001), lower global cognition (total z-score, p=0.049), a lower self-assessment of function (PAOF, p=0.044) and greater depression (CES-D, p=0.003). Further, the relationship between CCVL and depression (OR: 2.9 CI: 1.2-7.2) remained significant even after adjusting for BMI, sex at birth, daily alcohol use, and pre-ART plasma viral load. Similarly, the components used to calculate CCVL were not independently associated with depression or neurocognition.

**Conclusion**: A measure of the viral burden in the CNS due to local replication (i.e. CCVL) was more strongly associated with neuropsychiatric outcomes pre-ART than CSF or plasma viral load. These findings suggest that cognitive function and depression in ART-naive PWH is not only due to global changes in the immune and/or inflammatory environment, but may be directly related to compartmentalized replication in the CNS. These findings provide additional...
support for the need to fully suppress viral replication in the CNS during ART and the need to better understand the biology of HIV replication in the CNS.

### CELL-TYPE SPECIFIC GENOMICS AND TRANSCRIPTOMICS OF HIV IN THE BRAIN

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**Background:** Characterization of organ-specific reservoirs is critical as we look toward a functional cure for HIV. Due to the lack of reliable brain biomarkers of persistence and the difficulty of studying the human brain in vivo, there have not been in depth molecular studies of the brain reservoir. Viral integration is a key step in establishing the reservoir. Studies in T-cells have shown that HIV preferentially integrates into active, highly expressed regions of the genome; however, integration and its effects have not been studied in the brain.

**Methods:** Neuronal and glial nuclei were isolated using fluorescence activated cell sorting from postmortem frontal cortex samples provided by the Manhattan HIV Brain Bank. Integration site sequencing libraries were generated for neurons and glia for a total of 27 brains (n=6 HIV+, n=15 HIV+ non-encephalitis, n=6 HIV encephalitis [HIVE]). A subset of samples were submitted for 10X Chromium single nucleus RNA-seq (snRNA-seq).

**Results:** We identified 1,279 integration sites (IS), predominantly from the glial cell fraction of HIV cases. Glial IS were found preferentially in introns, gene dense regions, and active regions of the genome as marked by H3K37ac. Glial IS showed a stronger preference for integration into SINE repeat elements than T-cell IS and contain a significantly lower proportion of clonal (5% vs 18%, p<0.0001) and recurrent (13% vs 30%, p<0.0001) IS. Notably all IS were found to be in genes that were highly expressed in HIV microglia by snRNA-seq, and genes that were targeted for recurrent integration were expressed at the same level as non-recurrent genes. Differential expression analysis revealed that microglia with active viral transcription have elevated expression of core markers of microglial activation and decreased expression of markers of proliferation.

**Conclusion:** We see evidence for glial-specific patterns of IS selection. CPSF6 and LEDGF, the two proteins involved in IS targeting in T-cells, are not highly expressed in microglia, raising the possibility that there may be different mechanisms of integration in the brain. snRNA-seq also revealed that there are changes in activation and proliferation specifically within those microglia with active viral transcription. These findings coupled with the low proportion of clonal integration sites found in glia indicate that HIV infected microglia do not proliferate.

### PREDICTIVE FACTORS FOR HIV-1 CSF ESCAPE IN NEUROCOGNITIVE IMPAIRMENT

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**Background:** Among people with HIV (PWH) enrolled in the Neurocognitive Assessment in the Metabolic and Aging Cohort (NAMACO) study, we have observed a neurocognitive impairment (NCI) prevalence of 40%. In the current study, we examine the characteristics of patients with HIV viral escape in the cerebrospinal fluid (CSF).

**Methods:** We pooled data from NAMACO study participants and from patients attending a neuro-HIV platform in Switzerland. The NAMACO study is an ongoing, prospective, longitudinal, multicentre study of aging (≥45 years old) PWH enrolled in the Swiss HIV Cohort Study (SHCS). NAMACO participants in whom HIV-related NCI is diagnosed are invited to pursue investigations with a neurological examination, brain MRI and CSF analysis. The neuro-HIV platform is a multi-disciplinary full outpatient assessment at Lausanne University Hospital for PWH of any age, enrolled in the SHCS or not, in whom NCI is suspected. We analysed the demographic, clinical, immunological, neurocognitive and radiological characteristics of PWH who underwent lumbar puncture (LP) as part of the NAMACO study or the neuro-HIV platform between 1 March 2011 and 30 April 2019. CSF viral escape was defined as 1) the presence of quantifiable HIV RNA in the CSF at any level when plasma HIV RNA was suppressed or 2) CSF HIV RNA greater than plasma HIV RNA when the latter was detectable.

**Results:** Of 1166 PWH assessed, 287 underwent LP. The majority had suppressed plasma HIV RNA. CSF viral escape was observed in 29 patients (10%) of whom 18 (62%) had suppressed plasma HIV RNA and 11 (38%) had detectable plasma HIV RNA. Characteristics of patients were comparable whether they had CSF viral escape or not, including demographic profile, cardiovascular and metabolic comorbidities, time since HIV diagnosis (12 vs 16 years, p=0.4), median current CD4 count (558/mm, vs 611/mm, p=0.1) and median CD4 nadir (170/mm, vs 171/mm, p=0.7), antiretroviral CSF Penetration-Effectiveness score (7 vs 8 points, p=0.2), neurocognitive diagnosis based on Frascati criteria and presence of MRI abnormalities.

**Conclusion:** In this large pooled sample of PWH assessed for NCI, CSF viral escape occurred in 10% of patients. Patients with CSF viral escape presented no significant demographic, clinical, immunological, neurocognitive or radiological differences compared to patients without CSF escape. We conclude that LP remains the only reliable means of diagnosing HIV-1 escape in the CSF.

### ELUCIDATING THE ORIGINS OF HIV-1 IN THE CEREBROSPINAL FLUID

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**Background:** HIV-1 infection of the CNS has implications for both treatment and care strategies. Putative CNS reservoirs would likely be linked to sites of viral replication. HIV-1 RNA in the cerebrospinal fluid (CSF) can occasionally be genetically distinct from virus in the blood, indicating that the CNS can serve as a site for independent viral replication. However, the source of HIV-1 in the CSF from within the CNS is unknown and thus the sites of viral replication, and sites of potential reservoirs, remain to be determined.

**Methods:** Paired blood, CSF, and autopsy tissue samples from multiple brain regions were obtained from 5 participants enrolled in the NITC. These individuals were diagnosed with HIV-associated dementia, and no participant was taking ART at the time of death. HIV-1 genomes were extracted from blood/CSF (RNA) and brain tissues (DNA). The burden of HIV-1 DNA in various regions of the brain was quantified using ddPCR. Single genome amplification was performed on blood, CSF, and brain tissue samples in order to obtain full-length HIV-1 env sequences. Pseudotyped reporter viruses were generated from a total of 32 env genes cloned from 5 participants (22 from brain or CSF and 10 from blood plasma) and used in single-cycle entry assays to assess entry phenotype.

**Results:** In this cohort of 5 untreated people with HAD, the burden of HIV-infected cells varied across different regions of the brain, ranging from below the limit of detection to over 100,000 copies per million cells. In 3/5 participants, brain- and CSF-derived sequences were compartmentalized from blood-derived sequences. Overall, viral diversity present in the CSF represents much of the viral diversity found in the brain, but brain-specific lineages were also observed. In 2/5 participants, blood, CSF, and brain-derived sequences were intermingled, suggestive of a lack of independent viral evolution within the CNS. Compartmentalized CSF-brain Env proteins mediated efficient entry into cells expressing low densities of CD4 (M-tropic), whereas equilibrated Env proteins did not (RS T-tropic).

**Conclusion:** In this small, pilot study we observed that the viral diversity present in the CSF represents much, but not all, of the viral diversity present within various regions of the brain. Indeed, viral diversity and entry phenotype can vary across different regions of the brain in the same participant. Altogether, the CSF captures the majority of the genotypic and phenotypic properties of HIV-1 in the CNS of individuals with HAD.
PEMBROLIZUMAB TREATMENT IS ASSOCIATED WITH DECREASED CELL-ASSOCIATED HIV DNA IN CSF

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Background: Pembrolizumab is a monoclonal antibody against programmed death-ligand 1 (PD-L1) and is being evaluated as therapy for HIV infection. In the CNS, CA-HIV DNA is of particular interest due to its potential role in reservoir persistence. We evaluated the effects of pembrolizumab on immunologic markers such as the central nervous system (CNS), is a crucial step in any viral clearance strategy. We evaluated the effects of pembrolizumab on immunologic markers in the blood and cerebrospinal fluid (CSF) to test the hypothesis that this treatment may be used to target HIV reservoirs in the CNS.

Methods: HIV+ participants (n=6) were given a single dose of pembrolizumab in this phase I proof-of-concept study. CSF was collected at baseline, 3, and 24 weeks after dosing. Safety and tolerability were also monitored at these timepoints. All participants had a CD4 count of >350 cells/µL at baseline and were on ART with a plasma HIV RNA ≤ 40 copies/mL for at least 12 months prior to starting the trial. Multiparameter flow cytometry was obtained from 18.5mL of fresh CSF. HIV-1 DNA was quantified by limiting dilution PCR targeting a panel of fresh CSF. HIV-1 DNA was quantified by limiting dilution PCR targeting a panel of a region of env.

Results: Pembrolizumab administration was associated with a sharp decrease in cell-associated (CA)-HIV DNA levels in the CSF. CA-HIV DNA levels were a median of 46.1% lower at week 3 when compared to pre-treatment levels. At week 24, levels remained similar, a median of 71.7% lower when compared to week 3 (Fig. 1a). As expected, the proportion of CSF PD-1+ CD4+ and CD8+ T cells declined between baseline and week 3 and rebounded back to baseline levels at week 24 (Fig. 1b-c). Expression of other immune checkpoint points (PD-1, TIGIT, LAG3) appeared to be independent of PD-1 expression at week 24 (data not shown). Adverse events (AE) were minimal, with only one grade 2 AE of hypertriglyceridemia, and no grade 3 AEs.

Conclusion: A single dose of pembrolizumab was well-tolerated and effectively targeted PD-1 in the CSF. In most participants, pembrolizumab was associated with a reduction in CA-HIV DNA, highlighting its potential role in reservoir targeting studies. In the CNS, CA-HIV DNA is of particular interest due to its association with worse cognitive performance.

Figure 1. Pembrolizumab reduces cell-associated HIV DNA in cerebrospinal fluid (a), and measurements of PD-1 expression on T cells (b-c) confirm its effectiveness in the central nervous system.
neuropathic pain. re-establish a healthy gut microbiota and determine if this prevents or improves short chain fatty acids, are discussed. Future studies might test interventions to pro-inflammatory components and microbially-produced anti-inflammatory streptococcus to lachnospira, may contribute to prevalent neuropathic pain conditions, suggest that gut dysbiosis, particularly with DNP than in those without (t-test, p = 0.001). The Ruminococcus association is reported with note of the potential error in identification, as indicated by brackets. Furthermore, the log-

**Results:**
Participants were 267 PWH and 106 PWoH, 20.1% females, 45.3% nonwhite. Among PWH, median (interquartile range, IQR) nadir and current CD4 were 174 (21, 302) and 618 (448, 822) respectively; 90% were virally suppressed on antiretroviral therapy. PWH and PWoH did not differ with respect to microbiome diversity as indexed by Faith's phylogenetic diversity (PD). More severe neuropathic pain was associated with lower alpha diversity as indexed by Faith's PD (t = -0.158, p = 0.0043) in PWH, but not in PWoH. These relationships were not confounded by demographics or disease factors. In addition, the log-ratio of features identified at the genus level as (Ruminococcus) to Lachnospira was statistically significantly higher in PWH with DNP than in PWoH without DNP (t-test, p = 0.007). The Ruminococcus association is reported with note of the potential error in identification, as indicated by brackets. Furthermore, the log-ratio of Streptococcus features to Lachnospira features also was higher in PWH than in those without (t-test, p = 0.001).

**Conclusion:** Our results, in combination with previous findings in other neuropathic pain conditions, suggest that gut dysbiosis, particularly reductions in diversity and relative increases in the ratios of Ruminococcus and Streptococcus to Lachnospira, may contribute to prevalent neuropathic pain in PWH. Two candidate pathways for these associations, involving microbial pro-inflammatory components and microbially-produced anti-inflammatory short chain fatty acids, are discussed. Future studies might test interventions to re-establish a healthy gut microbiota and determine if this prevents or improves neuropathic pain.

**GUT DYSBIOSIS IN PEOPLE WITH HIV WHO HAVE NEUROPATHIC PAIN**

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**Background:** Gut dysbiosis, defined as pathogenic alterations in the distribution and abundance of different microbial species, is associated with neuropathic pain in a variety of clinical conditions such as nerve trauma, but this has not been explored in the context of neuropathy in people with PWH.

**Methods:** We assessed gut microbial diversity in PWH and people without HIV (PWoH), some of whom reported distal neuropathic pain (DNP), DNP was graded on a standardized, validated severity scale. The gut microbiome was characterized using 16S rRNA sequencing and diversity was assessed by phylogenetic tree construction. Songbird analysis was used to produce a multinomial regression model predicting counts of specific microbial taxa through metadata covariate columns.

**Results:** Participants were 267 PWH and 106 PWoH, 20.1% females, 45.3% nonwhite. Among PWH, median (interquartile range, IQR) nadir and current CD4 were 174 (21, 302) and 618 (448, 822) respectively; 90% were virally suppressed on antiretroviral therapy. PWH and PWoH did not differ with respect to microbiome diversity as indexed by Faith’s phylogenetic diversity (PD). More severe neuropathic pain was associated with lower alpha diversity as indexed by Faith’s PD (t = -0.158, p = 0.0043) in PWH, but not in PWoH. These relationships were not confounded by demographics or disease factors. In addition, the log-ratio of features identified at the genus level as (Ruminococcus) to Lachnospira was statistically significantly higher in PWH with DNP than in PWoH without DNP (t-test, p = 0.007). The Ruminococcus association is reported with note of the potential error in identification, as indicated by brackets. Furthermore, the log-ratio of Streptococcus features to Lachnospira features also was higher in PWH with DNP than in those without (t-test, p = 0.001).

**Conclusion:** Our results, in combination with previous findings in other neuropathic pain conditions, suggest that gut dysbiosis, particularly reductions in diversity and relative increases in the ratios of Ruminococcus and Streptococcus to Lachnospira, may contribute to prevalent neuropathic pain in PWH. Two candidate pathways for these associations, involving microbial pro-inflammatory components and microbially-produced anti-inflammatory short chain fatty acids, are discussed. Future studies might test interventions to re-establish a healthy gut microbiota and determine if this prevents or improves neuropathic pain.
underlying HIV neuropathogenesis in the central nervous system (CNS) will require relevant model systems. Microglia are resident myeloid cells of the brain that are readily infected by HIV and are likely to contribute to HAND. The purpose of this study was to define the most relevant microglial model systems for those working on HIV infection, replication, and pathogenesis in the CNS.

Methods: We evaluated two microglial model cell lines (C20, HMC3) and two sources of primary cell-derived microglia (monocyte-derived microglia [MMG] and induced pluripotent stem cell-derived microglia [iPSC-MG]) as model systems for studying HIV-microglia interactions. Cells were evaluated for relevant marker expression by flow cytometry and immunofluorescence microscopy. Gene expression analysis was performed and results compared between model systems and published microglial datasets.

Results: All four microglial model cells expressed typical myeloid and microglia-specific markers. Significant differences were observed upon gene expression profiling, however. MMG and iPSC-MG clustered closely with primary human microglial cells, while C20 and HMC3 exhibited marked differences. iPSC-MG and MMG expressed HIV-related genes in a manner closely resembling primary microglia. iPSC-MG and MMG were readily infected with R5-tropic HIV, while C20 and HMC3 required pseudotyping for infection. HIV replication dynamics and HIV-1 particle capture by Siglec-1 differed noticeably between MMG and iPSC-MG. In order to study HIV neuropathogenesis in a more CNS representative system, these findings are being extended to a three-dimensional (3D) iPSC-based cerebral organoid model incorporating iPSC-MG.

Conclusion: iPSC-MG and MMG were superior to transformed microglial cell lines in their similarity to authentic microglia, expression of HIV-relevant genes, and capacity to support HIV replication.

352 VALIDATION OF RAPID SEMIQUANTITATIVE LATERAL FLOW ASSAY FOR URINE TENOFOVIR

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Background: Objective measurements of antiretroviral adherence may be clinically useful for promoting interventions to improve prevention or treatment outcomes. In people receiving oral antiretrovirals containing tenofovir (TFV), recent adherence is correlated with the concentration of TFV in the urine. The purpose of this study was to validate our newly developed semi-quantitative LFA for urine TFV and compare its performance against LC-MS, ELISA and a commercial LFA reader.

Methods: We developed a semi-quantitative competitive lateral flow immunoassay (LFA) for TFV in urine, which classifies TFV levels as one of the three categories ‘adequate/low/undetectable’ by either optical scanner or visual scorecard. These three categories are indicated as TFV either above 1,500 ng/mL, between 150 ng/mL to 1,500 ng/mL, or below 150 ng/mL, and approximately correspond with the last oral dose having been taken 0 to 2, 2 to 4, or >4 days ago, respectively. We assessed the test using urine samples collected in a previous study of TFV pharmacokinetics following directly-observed tenofovir disoproxil fumarate (TFV-ds) administration. Samples were tested by LFA and liquid chromatography–mass spectrometry (LC-MS), as well as an enzyme-linked immunosorbent assay (ELISA) which we also developed. Test line intensities were scored visually 0 to 5 and quantitatively by optical LFA reader.

Results: 588 urine samples from 28 participants were measured, with TFV concentration ranging from <50 ng/mL to >23,200 ng/mL. LFA test line intensity was inversely proportional to TFV concentration, with a Spearman’s correlation coefficient of -0.91. Visual grading performed independently by two trained personnel were concordant within 1 grade for 96% of samples. The LFA sensitivity & specificity for classifying the 1,500 ng/mL threshold by LC-MS were 87% & 92%, respectively, for the average visual reads, and 95% & 96% when read by the reader. For the 150 ng/mL ‘undetectable’ threshold by LC-MS, the sensitivity and specificity were 84% & 89% (visual) and 87% & 95% (reader). For the ELISA, the sensitivities & specificities for the 1,500 ng/mL and 150 ng/mL thresholds by LC-MS were 92% & 90% and 90% & 93%, respectively.

Conclusion: Our rapid LFA test was sensitive and specific at classifying TFV concentrations into three clinically relevant ranges. These results support the feasibility of our rapid semi-quantitative urine test using either visual score or optical readout to assess recent ingestion of TFV for treatment or prevention.

353 IMPLICATION OF MEASURING URINE TENOFOVIR BY RAPID LATERAL FLOW ASSAY FOR DOSE RECENCY

Xin Niu, Derin Sevenler, Sandy Dossantos, Rebecca Sandlin, Mehmet Toner, Tim R. Cresssey, Paul K. Drain

Dose recency is correlated with time since last dosing. Benchmarks from this study will help guide and promote future adoption of LFA as a low-cost and easy-to-perform point-of-care (POC) test for PrEP/ART adherence monitoring and interventions.
Table 1: Proportions of Having a Time Since Last Dose Beyond Different Time Intervals Based on Different LFA Averaged Vision Scores and Optical Reader Readings Cutoffs.

<table>
<thead>
<tr>
<th>Hours since last dose</th>
<th>≤0.5</th>
<th>&gt;0.5</th>
<th>&gt;1.0</th>
<th>&gt;1.5</th>
<th>&gt;2.0</th>
<th>&gt;2.5</th>
<th>&gt;3.0</th>
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<td>≤24</td>
<td>93.4%</td>
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<td>100.0%</td>
<td>100.0%</td>
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<td>100.0%</td>
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</tr>
<tr>
<td>&gt;24</td>
<td>75.8%</td>
<td>96.3%</td>
<td>91.9%</td>
<td>97.4%</td>
<td>93.7%</td>
<td>95.7%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>&gt;72</td>
<td>60.8%</td>
<td>72.1%</td>
<td>82.6%</td>
<td>89.5%</td>
<td>98.3%</td>
<td>98.6%</td>
<td>100.0%</td>
<td>100.0%</td>
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</tr>
<tr>
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<td>75.7%</td>
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<td>96.9%</td>
<td>100.0%</td>
<td>100.0%</td>
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<td>62.5%</td>
<td>75.0%</td>
<td>53.3%</td>
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<td>62.5%</td>
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354 ESTABLISHING THE CUT-OFF FOR A URINE-BASED POINT-OF-CARE TEST FOR ADHERENCE TO TAF
Matthew Spinelli1, Dave Glidden2, Mary Morrow3, Samantha MalWhinney2, Kelly A. Johnson2, Hideaki Okochi1, Warren Rodrigues2, Guohong Wang2, Monica Gandhi1, Peter Anderson3
1University of California San Francisco, San Francisco, CA, USA, 2University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 3Abbott Labs, Abbott Park, IL, USA

Background: Urine drug-level monitoring can be used to measure adherence objectively to ART or PrEP in real time. Both tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) are metabolized to tenofovir (TFV). We previously developed an antibody-based immunoassay for tenofovir (TFV), and developed a point-of-care (POC) test to accurately exclude dosing in the last 5 days for TDF using a TFV cut-off in urine of 1500 ng/ml. Since plasma and urine levels of TFV are lower with TAF than TDF, a separate cut-off will be needed to assess TAF-based adherence. We modelled an optimal cut-off for urine TFV using TAF by using urine samples from a previously completed directly-observed therapy (DOT) study, TAF-DBS.

Methods: TAF-DBS randomized volunteers to 33%, 67%, or 100% adherence to TAF/emtricitabine and collected urine after 4 and 8 weeks of continuous dosing. Urine TFV levels were measured using liquid chromatography tandem mass spectrometry. An internal mixed-effects linear regression model evaluated possible cut-offs for a POC assay. The probabilities of being below a given cut-off at any time since the last dose were calculated from the model using the estimated mean, and individual and residual variation. The cut-off was optimized based on participant feedback that the assay should have high specificity for daily dosing.

Results: Thirty-six participants provided two urine samples each for this analysis (17 female, 7 Black, 6 Latinx). The estimated mean urine TFV concentration (ng/ml) at 24, 48, and 72 hours was 1530 (95% CI 1367-1712), 724 (95% CI 608-861), and 342 (95% CI 249-469) respectively. Dosing patterns were not associated with urine levels in models incorporating time since dosing (p=0.52). A tenofovir cut-off of 300 ng/ml optimized specificity for daily TAF dosing (98% probability; 95% CI: 96-100%), while maintaining high sensitivity (p=0.52). A tenofovir cut-off of 300 ng/ml optimized specificity for daily TAF dosing. Urine TFV levels were measured using liquid chromatography tandem mass spectrometry (LC/MS/MS) for participants receiving each TAF dose, combined across all adherence patterns.

Conclusion: A tenofovir cut-off of 300 ng/ml for a POC urine assay would accurately classify those with daily TAF dosing and exclude dosing within 5 days with high specificity. The interpretation of a negative test is clear: a participant would require urgent adherence counseling to avoid loss of virologic control on ART or decrease the risk of HIV acquisition on PrEP.

Table 1: Probability of urine TFV being below different cut-offs based on hours since last directly-observed dose in TAF-DBS

<table>
<thead>
<tr>
<th>Hours since last dose</th>
<th>100 ng/ml</th>
<th>300 ng/ml</th>
<th>500 ng/ml</th>
<th>700 ng/ml</th>
<th>1000 ng/ml</th>
<th>1500 ng/ml</th>
<th>(TDF cut-off)</th>
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<td>0%</td>
<td>1%</td>
<td>1%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>24</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>4%</td>
<td>8%</td>
<td>29%</td>
<td>49%</td>
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<td>36</td>
<td>0%</td>
<td>2%</td>
<td>7%</td>
<td>11%</td>
<td>17%</td>
<td>47%</td>
<td>67%</td>
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<td>48</td>
<td>1%</td>
<td>5%</td>
<td>15%</td>
<td>22%</td>
<td>32%</td>
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<td>82%</td>
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<td>32%</td>
<td>50%</td>
<td>81%</td>
<td>92%</td>
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<td>20%</td>
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<td>58%</td>
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<td>91%</td>
<td>97%</td>
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<td>84</td>
<td>14%</td>
<td>42%</td>
<td>96%</td>
<td>75%</td>
<td>83%</td>
<td>97%</td>
<td>99%</td>
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</table>

355 URINE TENOFOVIR CONCENTRATIONS ARE LOWER AMONG INDIVIDUALS TAKING TAF THAN TDF
Kelly A. Johnson1, Matthew Spinelli2, Xin Ni3, Dave Glidden2, Samantha MalWhinney3, Mary Morrow4, Hideaki Okochi1, Tim R. Cressy4, Paul K. Drain2, Monica Gandhi1, Peter Anderson3
1University of California San Francisco, San Francisco, CA, USA, 2University of Washington, Seattle, WA, USA, 3Columbia School of Public Health, Aurora, CO, USA, 4Chiang Mai University, Chiang Mai, Thailand, 5University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Background: A urine-based point-of-care (POC) immunoassay to measure urine tenofovir (TFV) levels for patients on tenofovir disoproxil fumarate (TDF) as an objective adherence metric has been developed. However, tenofovir alafenamide (TAF) is commonly used in HIV treatment and has been approved for pre-exposure prophylaxis (PrEP) among men who have sex with men. Since plasma TFV levels are nearly 10-fold lower with TAF than TDF in directly observed therapy (DOT) studies, we hypothesized that urine TFV levels would also be lower, with impacts for the POC assay. The purpose of this study was to leverage a TAF DOT study to: (1) determine urine TFV levels among individuals taking TAF at variable dosing intervals, and (2) compare them to a prior study of DOT TDF.

Methods: The TAF-DBS study randomized healthy HIV negative adult participants to take TAF 25mg/FTC 200 mg under DOT conditions at either 33, 67, or 100% of daily dosing levels for 12 weeks. Urine specimens were collected at 4 and 8 weeks. Urine TFV levels were quantified by liquid chromatography/tandem mass spectrometry (LC/MS/MS) for participants receiving each TAF dosing strategy. Urine levels were compared to those from individuals on TDF from the previously reported TARGET DOT study. In both TARGET and a prior analysis using TAF, time since dosing, rather than dosing pattern, determined TFV urine concentration; we thus present urine concentrations as time since last dose, combined across all adherence patterns.

Results: TAF-DBS included 36 participants (17 female, 7 Black, and 6 Latinx), each of whom provided two urine samples, with a median age of 29 (range 18-41) and a median estimated GFR of 98 ml/min (range 78-137). The median (IQR) urine TFV levels at 24, 48, and 72 hours after last TAF dosing were 1350 (766-2710), 497 (364-1160), and 254 (144-340) ng/ml, respectively, as compared to 6480 (3885-13550), 3045 (1948-4502), and 1210 (709-1832), respectively, with TDF in the TARGET study (Figure 1).

Conclusion: At each assigned dosing interval, urine TFV levels on TAF were approximately 80% lower than those from participants on TDF under DOT conditions, mirroring what has been observed in plasma with DOT. Given these results, a separate, lower TFV cut-off will be needed for any POC urine assay designed to assess TAF-based adherence on either ART or PrEP. Although the
cut-off will be lower for TAF than TDF in a POC adherence assay, the cut-off is still within range to develop a lateral flow assay and such efforts are underway.

356 HAIR MASS SPECTROMETRY IMAGING CAPTURES SHORT- AND LONG-TERM PrEP ADHERENCE CHANGES

Joseph Mwangi1, Nicole White1, Kelly Knudtson1, Amanda Poliseno1, Craig Sykes1, Lisa Hightow-Weidman1, Angela D. Kashuba1, Elias Rosen1  
1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA  
**Background:** Most adherence measures for antiretroviral (ARV) therapy require a blood sample, and none capture longitudinal daily adherence. We recently developed a noninvasive method for measuring daily adherence to emtricitabine (FTC)-based regimens in hair strands using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI), benchmarked by a directly observed therapy study. We also demonstrate daily FTC hair adherence classification for young men who have sex with men (YMSM) enrolled in the P3 (Prepared, Protected, empPowered) study who are engaged in, or initiating, preexposure prophylaxis (PrEP).

**Methods:** P3 volunteers (n=8, S1-8) reporting a range of recent adherence to PrEP at enrollment provided hair and blood samples at study initiation (T0) and after one month (T1). IR-MALDESI MSI measured FTC in the proximal 1cm (~30 days of growth) of hair strands (n=5) in 100 μm increments. Daily adherence was classified in composite longitudinal profiles. Analysis of FTC triphosphate (FTCtp) and tenofovir diphosphate (TFVdp) in dried blood spots was performed by a validated LC-MS/MS assay, IR-MALDESI and LC-MS/MS measures were compared by Spearman rank correlation (rs).

**Results:** IR-MALDESI analysis of P3 hair strands measured distinct patterns of FTC adherence at T0 and T1, which were categorized into four groups: consistently high adherence (Fig A); high adherence with occasional missed doses (Fig B); improved adherence after study initiation (Fig C); and, intermittent adherence (Fig D). Strong correlation in cumulative adherence was found between T1 TFVdp (inset) and a 60-day FTC average of concatenated T0 and T1 FTC profiles (rs=0.79, P=0.03). Agreement of recent adherence between FTCtp and the proximal 5-7 days of FTC adherence classification was variable since collecting hair by cutting close to the scalp doesn’t include the most recent days of growth. In each case, the combined 30-day adherence profiling of FTC in samples collected at T0 and T1 provided a granularity of day-to-day behavior not offered by the information derived from FTCtp/TFVdp concentrations.

**Conclusion:** Identifying patterns of long-term dosing behavior has important implications for the care of individuals using ARVs for treatment and prevention. IR-MALDESI MSI provides a daily assessment of PrEP adherence in hair, reflecting both short-term and long-term behavior.

357 HAIR MASS SPECTROMETRY IMAGING OF DAILY MARAVIROC ADHERENCE IN HPTN 069/ACTG 5305

Elias Rosen1, Nicole White1, Mac Gilliland1, Monica Gandhi1, Roy M. Gulick1, Angela D. Kashuba1  
1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 2Furman University, Greenville, SC, USA, 3University of California San Francisco, San Francisco, CA, USA, 4Weill Cornell Medicine, New York, NY, USA  
**Background:** Drug concentrations in hair can provide long-term measures of adherence. Classifying adherence by drug concentration may be less accurate for melanin-bound compounds like maraviroc (MVC) since darker hair colors can absorb greater amounts of drug. Using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) in the ENLIGHTEN study, we benchmarked longitudinal MVC profiles in hair following directly observed therapy (DOT) of daily and intermittent dosing. We scaled these measures with a melanin biomarker (pyrrole tricarboxylic acid, PTCA) to improve MVC adherence benchmarks. These criteria were applied in HPTN 069/ACTG 5305 (069/5305) to assess daily adherence to MVC-based PrEP regimens.

**Methods:** IR-MALDESI MSI measured MVC in the proximal 1-2cm and PTCA in the distal 0.5cm of the same 4 hair strands. Quantification of MVC in hair was
based on a calibration curve from incubated hair strands (LLOQ: 0.12 ng/mg hair). Benchmarking was performed in samples from 12 volunteers undertaking 28-day phases of daily and then intermittent (0x, 1x, or 3x/wk; n=4 in each group) MVC dosing in the ENLIGHTEN study. MVC dose-frequency benchmarks were identified in longitudinal profiles and normalized by PTCA, with an adherence threshold determined by an ROC curve. For 069/5305, MVC was measured by LC-MS/MS and IR-MALDESI in 32 samples from 19 individuals over matched segment lengths of 1 cm (~1 month of growth).

**Results:** Overlapping MVC dose-frequency ranges in ENLIGHTEN DOT samples (Fig. A) were resolved by PTCA normalization (Fig. B) [MVC/PTCA: daily median (interquartile range) 0.75 (0.44-0.85); 3x/wk 0.25 (0.14-0.33); 1x/wk 0.08 (0.02-0.14); 0x/wk 0.0 (0.0-0.01)]. A threshold of MVC/PTCA=0.35 was selected to classify daily adherence with 75% sensitivity and 100% specificity. Month-long adherence patterns were evaluated for 069/5305 samples (Fig. C). Strong correlation was found between IR-MALDESI and LC-MS/MS MVC concentrations (Fig. D; Spearman’s rho=0.59, P=0.007). MVC/PTCA yielded unambiguous adherence classification relative to MVC alone (Fig. E), indicating only 8/19 individuals adhered to a daily regimen throughout the prior month.

**Conclusion:** Hair color is an important factor for accurate adherence classification of MVC, and likely other antiretrovirals with similar physicochemical properties. Long-term, daily adherence classification of PTCA-scaled MVC was demonstrated by IR-MALDESI MS1 and found less than expected 069/5305 adherence.

**Table 1. Individual plasma and CSF data after 4 weeks with DOR=PTC/TAFF**

<table>
<thead>
<tr>
<th>ID</th>
<th>HIV RNA plasma copies/ml</th>
<th>HIV RNA CSF copies/ml</th>
<th>Total plasma DOR ng/ml</th>
<th>Unbound plasma DOR ng/ml</th>
<th>Total CSF DOR ng/ml</th>
<th>Unbound CSF DOR ng/ml</th>
<th>MVC/PTCA</th>
<th>wDORCSF/week</th>
<th>wDORCSF/week</th>
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<tbody>
<tr>
<td>1</td>
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<td>&lt;20</td>
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<td>47.1</td>
<td>163.7</td>
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<td>0.59</td>
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<td>44.6</td>
<td>0.20</td>
<td>1.10</td>
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**359 PERSISTENT HIV TRANSCRIPTION AND VARIABLE ARV LEVELS IN LYMPH NODES DURING ART**

Courtney V. Fletcher, Eugene Kroon, Timothy Schacker, Suteeraporn Pinyakorn, Nicolas Chomont, Suthat Chottanapund, Peeriyta Pruesaksawat, Khunthalee Benjapornpong, Suppaneen Buranapradit, Jintana Ananworanich, Sandhya Vasani, Denise C. Hsu, for the RV254/SEARCH 010 Study Group

1 University of Nebraska Medical Center, Omaha, NE, USA, 2SEARCH, Institute of HIV Research and Innovation, Bangkok, Thailand, 3University of Minnesota, Minneapolis, MN, USA, 4US Military HIV Research Program, Silver Spring, MD, USA, 5Centre de Recherche du CHUM, Montreal, Canada, 6Chulalongkorn University, Bangkok, Thailand, 7University of Amsterdam, Amsterdam, Netherlands

**Background:** The ability of antiretroviral (ARV) drugs to penetrate and suppress viral replication in tissue reservoir sites is critical for HIV remission. We evaluated ARV levels and their impact on HIV transcription in lymph nodes (LN).

**Methods:** This was a sub-study involving participants of the RV254/SEARCH010 Acute HIV Infection Cohort in Bangkok, Thailand. Group 1 (n=6) initiated and continued ARVs with 2 NRTI, dolutegravir (DTG) and maraviroc (MVC). Group 2 (n=12) initiated ARVs on 2 NRTI plus efavirenz and were switched to 2 NRTI plus DTG. HIV RNA+ and DNA+ cells were measured by RNAscope®. Cell-associated HIV RNA and total HIV DNA were measured by PCR. ARV levels were measured by liquid chromatography, tandem mass spectrometry.

**Results:** Participants median age 27 yrs, were all MSM. At LN biopsy all had plasma HIV-RNA <20 copies/mL. Group 2 had longer durations of ARV (median 125
was similar to the partition coefficients of other organs, consistent with TFV’s volume of distribution (~0.8 L/kg).

**Conclusion:** Our novel PBPK model is the first to predict TFV concentrations in lymph nodes of NHPs and humans. A permeability-limited lymph node model and KpLN = 1 accurately captures TFV’s rapid diffusion between plasma and tissue, which is expected based on its low lipophilicity, molecular weight, and minimal protein binding. Our PBPK model can be used to describe the lymph node distribution of ARVs with similar properties (FTC, TCT), or to test new formulations aimed at increasing lymphatic penetration.

### 361 PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF TFV AND TAF FOR PREP IN FORESKIN TISSUE

**Carolina Herrera**1, Laura Else2, Sujan D. Penchala3, Azure-Dee A. Pillay2, Thabiso B. Seiphethlo1, Limakatso Lebina1, Jordi Niubo4, Erin M. Scholz1, Sujan D. Penchala1, Arkaitz Imaz1, Daniel Tiraboschi5, Sofía Scevola5, Saye Khoob1

**1Imperial College London, London, UK, 2University of Liverpool, Liverpool, UK, 3University of Cape Town, Cape Town, South Africa, 4University of the Witwatersrand, Johannesburg, South Africa, 5Gilead Sciences, Inc, Foster City, CA, USA, 6Guys and St Thomas’ NHS Foundation Trust, King’s College London, London, UK**

**Background:** Pre-exposure prophylaxis (PrEP) studies have focussed predominantly on the efficacy in female reproductive and colorectal tracts, with limited research on the efficacy of PrEP candidates in the male genital tract. We assessed the ex vivo pharmacological profile of Tenofovir (TFV) and Tenofovir alafenamide (TAF) in foreskin tissue to inform the design of the CHAPS oral PrEP trial (NCT03986970).

**Methods:** Foreskin specimens were obtained with signed informed consent from HIV-negative males who volunteered routine medical circumcision. Inner mucosal and outer skin were cut in explants and exposed to serial dilutions of TFV or TAF for 1h prior to addition of HIV-1-83 Bal at a high (HVT) or low viral titre (LVT) for 2h. Infection was assessed at different time points during 15 days of culture by measurement of p24 in culture supernatants. TFV and TAF concentrations were measured in tissue, culture supernatants and dosing and washing solutions using LC-MS methods.

**Results:** Dose-response curves were obtained for both drugs against the two viral titres tested with greater inhibitory potency observed against LVT. Inhibitory equivalency mimicking oral dosing was defined between 1 mg/ml of TFV and 15 μg/ml of TAF against HVT for the dosing post-ex vivo challenge included in the design of CHAPS trial. Concentrations of TFV-DP in foreskin explants were at least 5 times higher after ex vivo dosing with TAF vs. TFV. Statistically significant negative linear correlations were observed between explant TFV-DP levels and KpLN values (r=-0.6696, P=0.0002 for TFV and r=-0.6698, P=0.0001 for TAF).

**Conclusion:** Pre-clinical evaluation of TAF reveals greater potency than TFV against penile HIV transmission. Ex vivo dose-calibration studies in human foreskin explants can be used as surrogate for in vivo studies to compare doses and preventive agents to be included in clinical trials.

### 362 DORAVIRINE CONCENTRATIONS AND HIV-1 RNA SUPPRESSION IN MALE AND FEMALE GENITAL FLUIDS

**Sofia Scevola**1, Arkaitz Imaz2, Mackenzie L. Cottrell3, Jordi Niubo4, Juan M. Tiraboschi3, Sandra Morenilla1, Irene Soriano1, Angela D. Kashuba1, Daniel Podzamczer1

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**Background:** Residual HIV replication in reservoirs might contribute to immune activation and inflammation and constitutes a barrier to eradication. Antiretroviral distribution in male and female genital tract is required to suppress HIV replication within these compartments. We determined doravirine concentrations and HIV-1 RNA in blood plasma (BP), seminal plasma (SP) and cervicovaginal fluid (CVF) of HIV-1-infected adults receiving ART with doravirine plus emtricitabine/tenofovir alafenamide (DOR+FTC/TAF).

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153wks, p<0.001) and DTG use (median 63wks, p=0.002) compared to group 1 (median 44wks, Table). Feibie stage, HIV viral load, CD4 and CD8 T cell counts at baseline and at LN biopsy were not different between groups. Median PBMC levels (fmol/10^6 cells) were: TFV-DP, 3.2; 3TC-TP, 8.39; CBV-TP, 4.5; LN cellular levels (Table) of TFV-DP, 3TC-TP, DTG and MVC were all measured in all participants; CBV-TP was quantifiable in 4/14 (29%) vs 100% of PBMC samples. Median ratios of LN cellular to in-vitro inhibitory levels (IC_{50} or 90) were: TFV-DP, 1.8; 3TC-TP, 4.1; CBV-TP, 0.5 (in n=9 with quantifiable levels); DTG, 0.8; MVC, 38.8. Ongoing viral expression was detected by RNAscope® in all participants. There were trends for lower RNA+ (median of 71530 vs 97950 cells/g, p=0.111) and DNA+ cells (median 169137.5 vs 303389 cells/g, p=0.512) in group 1 vs 2. PBMC levels of cell-associated HIV RNA and total HIV DNA were not different between groups.

**Conclusion:** PBMC levels of TFV-DP, 3TC-TP and CBV-TP were consistent with literature values, as were LN levels of TFV-DP and 3TC-TP. This is the first report of LN penetration of CBV-TP and MVC. CBV-TP LN levels were commonly not quantifiable and <10^4 copies/mL.
**Methods:** This prospective study included 15 male and 15 female HIV-1-infected, virologically suppressed adults on stable ART. ART was switched to DOR+ FTC/TAF. After 8 weeks, total and protein-unbound DOR concentrations were determined at the end of a dosing interval (C24h) in paired SP/CVF and BP samples. HIV-1 RNA was evaluated in SP/CVF and BP samples at baseline and week 8. Validated liquid chromatography-tandem mass spectrometry (‘LC-MS/MS’) was used to quantify DOR concentrations, and HIV-1 RNA was determined by real-time PCR (Abbott). Data are presented as median (range).

**Results:** 15 males and 14 females completed the study and were included in the analysis. Age was 41 years (23–62), time on ART 109 months (16–305) and CD4 count 791 cells/µl (329–1926). At baseline, all subjects had HIV-1 RNA <40 copies/mL in SP and CVF samples. Eight weeks after switching to DOR+ FTC/TAF, total DOR C24h was 127 ng/mL (31.2–272) in SP and 506 ng/mL (200–961) in CVF, corresponding to 35% and 106% of total DOR concentrations in BP, respectively. DOR protein-unbound fractions were 82.2% in SP and 63.5% in CVF. Protein-unbound DOR C24h was 104 ng/mL (27–218) in SP and 312 ng/mL (138–562) in CVF (Table 1). Thus, median protein-unbound DOR C24h in SP and CVF were 20.4-fold and 61.2-fold above the half-maximal effective concentration (EC50) for wild-type HIV-1 (5.1 ng/mL). At week 8, all individuals maintained HIV-1 RNA suppression <40 copies/mL in BP and genital fluid samples with the exception of one male with detectable HIV-1 RNA (263 copies/mL) in SP despite a high DOR concentration in this compartment (protein-unbound C24h 104 ng/mL).

**Conclusion:** Protein-unbound DOR concentrations in SP and CVF highly exceeded the EC50 value for wild-type HIV-1 in all individuals. DOR+ FTC/TAF seems effective to maintain HIV-1 RNA suppression in SP and CVF.

**Table 1: Corovine concentrations (Cov) in blood plasma, seminal plasma and cervicovaginal fluid**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Trough Time</th>
<th>% Achieving Cov in SP</th>
<th>% Achieving Cov in CT</th>
<th>% Achieving Cov in VT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>24 hr periclol F5 dose</td>
<td>23.5 hr (first dose)</td>
<td>88.2</td>
<td>78.9</td>
</tr>
<tr>
<td>0 hr, 36 hr periclol</td>
<td>35.5 hr (first dose)</td>
<td>92.3</td>
<td>69.3</td>
<td>95.8</td>
</tr>
<tr>
<td>0 hr, 48 hr periclol, TTSS dosing</td>
<td>47.5 hr (first dose)</td>
<td>94.5</td>
<td>58.4</td>
<td>83.6</td>
</tr>
</tbody>
</table>

**2x/week dosing**

| 83.5 hr (steady state) | 99 | 65 |

**4x/week dosing**

| 41.5 hr (steady state) | 99 | 95 |

**7x/week dosing**

| 23.8 hr (steady state) | 100 | 100 |

**IPERGAY (double dose 24 hr pre-coitosis, followed by single dose every 24 hours post-coitosis)**

| 81 (24 hr) | 98 (24 hr) | 98 (24 hr) | 100 (24 hr) |

*As reported in PMC4907409; female genital tract concentrations were reported as the composite of CT and VT as “lower female genital tract tissue.”

**HIGH LUNG LEVELS OF ACTIVE TRIPHOSPHATE PREDICTED WITH ORAL AT-527 IN COVID PATIENTS**

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**Background:** AT-527 is a guanosine nucleotide prodrug with potent in vitro antiviral activity against flaviviruses and coronaviruses including SARS-CoV-2 (EC50 = 0.5 µM), the virus responsible for COVID-19. AT-527 exhibits a unique mechanism of action predominantly targeting the NiRAN function of the SARS-CoV-2 polymerase. The clinical safety to date and in vitro potency of AT-527 prompted evaluation of this drug candidate in patients with COVID-19. The purpose of this study was to assess the safety and pharmacokinetics (PK) of AT-527 dosed 550 mg twice a day (BID) in healthy subjects and to predict human lung exposure of the active triphosphate metabolite AT-9010.

**Methods:** Twenty healthy subjects were randomized 1:1 to receive orally AT-527 550 mg BID or matching placebo for 5 days. Safety assessments included adverse events (AEs), vital signs, electrocardiograms (EGGs) and standard safety laboratory tests. Intensive PK sampling was performed after the first and last two doses and assayed for plasma AT-511, the free base form, and metabolites AT-527 550 mg BID or matching placebo for 5 days. Safety assessments included adverse events (AEs), vital signs, electrocardiograms (EGGs) and standard safety laboratory tests. Intensive PK sampling was performed after the first and last two doses and assayed for plasma AT-511, the free base form, and metabolites AT-527 550 mg BID or matching placebo for 5 days. Safety assessments included adverse events (AEs), vital signs, electrocardiograms (EGGs) and standard safety laboratory tests. Intensive PK sampling was performed after the first and last two doses and assayed for plasma AT-511, the free base form, and metabolites AT-527 550 mg BID or matching placebo for 5 days. Safety assessments included adverse events (AEs), vital signs, electrocardiograms (EGGs) and standard safety laboratory tests.

**Results:** AT-527 was well tolerated with no discontinuations, serious AEs, clinically significant changes in vital signs or ECGs based on still blinded safety data. AT-511 was rapidly absorbed followed by fast and extensive metabolic conversion to an L-alanyl intermediate metabolite AT-551 and ultimately intracellular AT-9010, reflected by plasma AT-511, the free base form, and metabolites including AT-273, the guanosine nucleoside metabolite, a surrogate for intracellular AT-9010.

**Conclusions:** AT-527 was well tolerated with no discontinuations, serious AEs, clinically significant changes in vital signs or ECGs based on still blinded safety data. AT-527 was well tolerated with no discontinuations, serious AEs, clinically significant changes in vital signs or ECGs based on still blinded safety data. AT-527 was well tolerated with no discontinuations, serious AEs, clinically significant changes in vital signs or ECGs based on still blinded safety data.
INTTEGRATED DMPK ALGORITHM FOR THE PREDICTION OF ARV DDI MAGNITUDE

Sandra Grañana-Castillo1, Fazila S. Bunglawala1, Nicolas Cottura1, Asangaedem Akpan1, Rachel Bearon1, Saye Kho1, Marco Siccardi1
1University of Liverpool, Liverpool, UK

Background: The prevalence of potential drug-drug interactions (DDIs) is about 29% in people living with HIV (PLWH) undergoing treatment, which can impact the overall management of therapies. Only a minority of potential DDIs have actually been characterised in clinical studies; many DDIs remain unexplored clinically or cannot be studied due to ethical constraints. In most cases, the evaluation of DDIs is supported only by the individual judgment of the prescriber or expert opinion. Computer modelling tools can support the prediction of potential DDIs, providing a quantitative estimate of DDI magnitude. The aim of this study was to develop a quantitative algorithm for the prediction of enzymatic mediated induction DDIs of antiretrovirals (ARVs).

Methods: In vitro drug metabolism data for 73 drugs across multiple disease areas were integrated to provide a comprehensive description of the mechanisms underpinning induction DDIs, including: in vitro drug metabolism and pharmacokinetics (DMPK) data, transporter specificity and induction potential of metabolic enzymes. For each DDI substrate an in vitro metabolism metric (IVMM) was calculated through the integration of the fractions metabolised (Fm) by each hepatic enzyme multiplied by the inducer effect (E) for the corresponding enzyme isoform. Rifampicin was selected as a DDI perpetrator and a multiple linear regression model was generated to identify predictors of the DDI magnitude.

Results: The predicted area under the curve (AUC) ratios (with vs without rifampicin) obtained in our model were in agreement with the AUC ratios observed in clinical studies (Figure 1). Three independent in vitro variables were retained: IVMM, fraction unbound in plasma (Fu) and substrate specificity for the OATP1B1 transporter. All ARV (n = 11) DDI predictions were within 2-fold of the clinical data, respectively.

Of all 73 drugs included in the algorithm, 42%, 70% and 88% were within 1.25-fold (0.8-1.25), 1.5-fold (0.66-1.5) and 2-fold (0.5-2) of the clinical data, respectively.

Conclusion: Management of DDIs in PLWH is challenging, especially in an ageing cohort with accumulating multiple comorbidities and polypharmacy. This model provides a fit-for-purpose tool to predict potential DDIs, utilising accessible in vitro data which could prove advantageous in early drug development and potentially help facilitate a more rational design of clinical trials.

EXOGENOUS HORMONE PHARMACOKINETICS IN TRANSGENDER ADOLESCENTS RECEIVING ORAL TDF/FTC

Jenna L. Yager1, Kristina Brooks1, Jennifer Brothers1, Daniel Reiden1, Meena Malhotra1, Carrie Glenny1, Kathleen Mulligan1, Raphael J. Landonova2, Lucas Ellison1, Lane Bushman1, Jennifer Kiser1, Peter Anderson1, Sybil Hosek2
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2Stroger Hospital of Cook County, Chicago, IL, USA

Background: Transgender women (TW) and transgender men (TM) are at an increased risk of HIV infection, and would therefore benefit from the use of tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) as pre-exposure prophylaxis (PrEP). Although there are currently no data available regarding the pharmacokinetics of TDF/FTC among TW, limited data exist for TM and adolescents. This lack of information is a major acceptability concern for these populations. This study assessed whether estradiol pharmacokinetics in TW, and testosterone pharmacokinetics in TM were altered with daily PrEP use.

Methods: Adolescent TW and TM ages 15-24 years, who were HIV-uninfected and receiving a stable cHSiT dose for ≥ 1 month or 3 consecutive doses, were enrolled. Participants received directly observed daily TDF/FTC for 30 days. Serum was collected for estradiol (TW) or testosterone (TM) concentrations at baseline (5-7 timepoints, dependent upon cHSiT dosing schedule) and after 2-3 weeks of daily TDF/FTC dosing. Estradiol and total testosterone were quantified using LC/MS/MS and free testosterone with equilibrium dialysis (Brigham Research Assay Core Laboratory, Boston, MA). Area under the curve (AUC) and maximum concentration (Cmax) were calculated using noncompartmental methods. Results were log-transformed and compared using a paired t-test.

Results: Twenty-four TM and 25 TW were included. Testosterone was received intramuscularly by 12 (50%) TM and subcutaneously by 12 TM (50%). Estradiol was received orally by 13 (52%) TW and subcutaneously by 12 TW (48%). Eighteen (72%) TW were also receiving spironolactone. For TM, median (range) age was 21 (17-24) years, creatinine clearance (CrCl) was 101.5 (71-279) ml/min, and weight was 58.9 (47.8-129.5) kg. For TW, median (range) age was 20 (16-24) years, creatinine clearance (CrCl) was 101.5 (71-279) ml/min, and weight was 58.9 (47.8-129.5) kg. For both TW and TM, AUC of TDF and FTC were within 2-fold of clinical data, and testosterone pharmacokinetics in TM were altered with daily PrEP use.

Conclusion: TDF/FTC among HIV-uninfected adolescents did not significantly alter serum estradiol pharmacokinetics in TW, or free and total testosterone pharmacokinetics in TM. These data should be reassuring to persons in the transgender community with concerns about cHSiT during PrEP.

Table 1. Geometric mean AUC0-τ and Cmax serum estradiol and testosterone

<table>
<thead>
<tr>
<th></th>
<th>TW AUC0-τ (ng·h/ml)</th>
<th>Baseline</th>
<th>PrEP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-τ</td>
<td>(9416, 21,547)</td>
<td>(9169, 18,182)</td>
<td>0.867</td>
<td>0.0164</td>
</tr>
<tr>
<td></td>
<td>(4115, 21,547)</td>
<td>(9169, 18,182)</td>
<td>0.867</td>
<td>0.0164</td>
</tr>
<tr>
<td></td>
<td>(324, 507)</td>
<td>(183, 440)</td>
<td>0.849</td>
<td>0.2148</td>
</tr>
</tbody>
</table>

TVF-DP AND FTC-TP IN PBMC AMONG TRANSGENDER ADOLESCENTS RECEIVING DAILY TDF/FTC

Jenna L. Yager1, Kristina Brooks1, Jennifer Brothers1, Daniel Reiden1, Meena Malhotra1, Carrie Glenny1, Kathleen Mulligan1, Raphael J. Landonova2, Lucas Ellison1, Lane Bushman1, Jennifer Kiser1, Peter Anderson1, Sybil Hosek2
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2Stroger Hospital of Cook County, Chicago, IL, USA

Background: Transgender women (TW) and transgender men (TM) have historically been underrepresented in pre-exposure prophylaxis (PrEP) clinical trials. In vitro drug metabolism data for 73 drugs across multiple disease areas were integrated to provide a comprehensive description of the mechanisms underpinning induction DDIs, including: in vitro drug metabolism and pharmacokinetics (DMPK) data, transporter specificity and induction potential of metabolic enzymes. For each DDI substrate an in vitro metabolism metric (IVMM) was calculated through the integration of the fractions metabolised (Fm) by each hepatic enzyme multiplied by the inducer effect (E) for the corresponding enzyme isoform. Rifampicin was selected as a DDI perpetrator and a multiple linear regression model was generated to identify predictors of the DDI magnitude.

Methods: In vitro drug metabolism data for 73 drugs across multiple disease areas were integrated to provide a comprehensive description of the mechanisms underpinning induction DDIs, including: in vitro drug metabolism and pharmacokinetics (DMPK) data, transporter specificity and induction potential of metabolic enzymes. For each DDI substrate an in vitro metabolism metric (IVMM) was calculated through the integration of the fractions metabolised (Fm) by each hepatic enzyme multiplied by the inducer effect (E) for the corresponding enzyme isoform. Rifampicin was selected as a DDI perpetrator and a multiple linear regression model was generated to identify predictors of the DDI magnitude.
trials. Recently, a few studies have sought to address a potential drug-drug interaction between PreP and cross-sex hormone therapy (cSHt), but data remained limited, particularly among adolescent transgender individuals and TM.

**Methods:** This was a prospective study conducted among adolescent TW and TM. Participants were HIV-uninfected, between 15–24 years of age, and were receiving a stable dose of cSHt. They received 30 days of directly observed (DOT) daily tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC). PBMC were collected weekly for 4 weeks, at random times post-dose, and after 2–3 weeks of dosing, PBMC were collected at 0 (pre-dose), 4, and 24-hours.

**Intracellular tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) were determined using LC-MS/MS.** Average TFV-DP and FTC-TP in PBMC were calculated using noncompartmental methods. Results were then log-transformed and compared between TM and TW, and compared against TFV-DP and FTC-TP from historical DOT cohorts.

**Results:** Participants (24 TM, 26 TW) were included in this analysis. Among TW vs. TM, mean (SD) age (20.2 ± 2.3 vs. 20.2 ± 2.3 years), CrCl (13.8 [34.4] vs. 117 [48.4] ml/min), and weight (69 [11.9] vs. 69 [21.4] kg) were similar. Geometric mean Cavg TFV-DP and FTC-TP in PBMC are listed in the table. TM had 34% higher TFV-DP in PBMC vs. TW (GMR [95% CI]: 1.34 [1.002, 1.796], p = 0.0485) and 56% higher FTC-TP in PBMC vs. TW (GMR [95% CI]: 1.44 [1.14, 1.83], p = 0.0032). For comparison, values of TFV-DP and FTC-TP from historical DOT studies with 100% adherence ranged from 36.3–71.2 fmol/10^6 cells and 2.2–5.2 fmol/10^6 cells.

**Conclusion:** TM had higher TFV-DP and FTC-TP in PBMC vs. TW, consistent with previously-reported higher plasma TFV and FTC exposures. TFV-DP and FTC-TP in both populations were in line with previous DOT studies, suggesting no change in dosing is needed for PreP utilization in TM and TW to achieve expected concentrations.

**Table 1: Average concentrations of tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) at steady state**

<table>
<thead>
<tr>
<th>N</th>
<th>TFV-DP (fmol/10^6 cells)</th>
<th>FTC-TP (fmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>64.5 (55.5, 74.9)</td>
<td>5.11 (4.49, 5.81)</td>
</tr>
<tr>
<td>24</td>
<td>75.1 (62.5, 89.7)</td>
<td>6.19 (5.0, 7.22)</td>
</tr>
<tr>
<td>26</td>
<td>56.0 (44.2, 70.5)</td>
<td>4.28 (3.56, 5.15)</td>
</tr>
</tbody>
</table>

**Background:** Subdermal progestin-releasing implants, containing either etonogestrel (ENG) or levonorgestrel (LNG), are highly effective, long-acting, reversible contraceptive options. EFV-based ART significantly reduces the ENG and LNG exposure from both available implants, contributing to contraceptive failures. Alternative implant-ART combinations are needed but have been widely evaluated. Our objective was to characterise the pharmacokinetics (PK) of ENG and LNG, each released from a subdermal implant, over 24 weeks in women with HIV onrilpivirine (RPV)-based ART.

**Methods:** Two separate, parallel group, non-randomised, PK studies evaluating either ENG (ENG study) or LNG (LNG study) were conducted in women receiving RPV-based ART. Participants were aged 18 to 45 years and virologically either ENG (ENG study) or LNG (LNG study) were conducted in women receiving RPV-based ART.

**Results:** All participants were Black African females. Both control groups had higher mean baseline body weight (66 and 69 kg) compared to the RPV groups (60 and 55 kg) for the ENG and LNG studies, respectively. ENG and LNG concentrations and associated comparison with historical data are indicated in the Table. At week 24, both ENG and LNG were modestly higher in the RPV group compared to each control group (ENG: 1.18 (1.00 – 1.35); LNG: 1.28 (0.99 – 1.52)). One participant in the ENG study had grade 3 weight gain; no grade 3 or greater AEs were reported in the LNG study or in either control group.

**Conclusion:** Over the 24-week study period, concentrations of ENG and LNG from a contraceptive implant were modestly higher, but not clinically significant in women receiving RPV-based ART compared to the historical control groups. Our results support that both the ENG and LNG implant are effective contraceptive options for women receiving RPV-based ART.
Efficacy and PK of Dolutegravir 50 mg QD with Food Versus 50 mg BID with Rifampicin

Thornthun Uaphonphosungkit,1 Sivaporn Gatechompol,1 Jirachaya Sophonphan2, Stephen J. Kerr3, Hay Mar Su Lwin4, Win Min Han3, Sasawimon Ubolym1, Prachai Chayalang4, Charanmor Phothidokmai5, Yong Soon Cho6, Jae Gook Shin6, Anucha Avilingsanon7,8 for the HIV NAT 234 Study

Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, Tuberculosis Research Unit, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, ‘Center for Personalized Precision Medicine of Tuberculosis, Inje University College of Medicine, Busan, Korea, Republic of Korea, HIV-NAT, Thai Red Cross AIDS Research Centre, Bangkok, Thailand, Biostatistics Excellence Centre, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Background: Concurrent use of rifampicin (RIF) and dolutegravir (DTG) reduces DTG exposure, thus, DTG 50 mg twice-daily is currently recommended. Food increased DTG concentrations in healthy volunteers by 33 – 66%. We therefore investigated the effect of RIF on DTG exposure when dosed at 50 mg once daily (QD) compared to food (study arm; TLD 1 pill/day) or DTG 50 mg twice-daily (control arm; TLD 1 pill plus additional DTG). Intensive PK was scheduled at week 4. Blood samples were collected pre-dose, 1, 2, 4, 6, 12, and 24-hour post-dose (by study arm). HIV-RNA, liver and renal function tests were monitored. DTG concentrations were determined by validated LC-MS/MS methods, at the end of each treatment period (steady-state) at the end of dosing interval (Cmin) and at 2-6 h (start and after drug-dose escalations). Seventeen patients were enrolled in this study but, due to high incidence of liver toxicity only 4 patients completed the protocol.

Methods: We conducted a single-center, open-label study in Bangkok, Thailand. TB/HIV coinfected adults, ART naive, stable on RIF containing regimen for drug-susceptible TB were randomly assigned to receive DTG 50 mg OD with food (study arm; TLD 1 pill/day) or DTG 50 mg twice-daily (control arm; TLD 1 pill plus additional DTG). Intensive PK was scheduled at week 4. Blood samples were collected pre-dose, 1, 2, 4, 6, 12, and 24-hour post-dose (by study arm). HIV-RNA, liver and renal function tests were monitored. DTG concentrations were determined by validated LC-MS/MS methods, at the end of each treatment period (steady-state) at the end of dosing interval (Cmin) and at 2-6 h (start and after drug-dose escalations). Seventeen patients were enrolled in this study but, due to high incidence of liver toxicity only 4 patients completed the protocol.

Results: Overall plasma and intra-PBMCs Cmin of DRV were lower than in control arm but similar to what reported in literature. DRV intra-PBMC/plasma Cmax ratios were 1.07 (0.77-1.46) and 1.07 (0.77-1.46), respectively (Table 1). Cmin GMR in the study arm was 0.3 (0.26-0.36), but 83% and 94% of study and control arm participants had HIV-RNA <40 copies/mL. Both arms were well-tolerated.

Conclusion: Although there were substantial reductions in DTG concentrations when co-administered with RIF, Cmin levels were mostly above the protein-binding-adjusted IC50 of 0.064 μg/mL and majority of participants (>80%) had VL suppression at week 12.

Table 1. Summary of Pharmacokinetic Parameter Estimates across Study Treatments

<table>
<thead>
<tr>
<th>PK Parameter (Mean ± SE)</th>
<th>QD-BD</th>
<th>TLD</th>
<th>QD-BD</th>
<th>TLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>1800 (110)</td>
<td>1500 (100)</td>
<td>1800 (110)</td>
<td>1500 (100)</td>
</tr>
<tr>
<td>AUC0–τ (ng*h/mL)</td>
<td>35000 (2500)</td>
<td>30000 (2000)</td>
<td>35000 (2500)</td>
<td>30000 (2000)</td>
</tr>
<tr>
<td>Cmin (ng/mL)</td>
<td>75 (5)</td>
<td>60 (5)</td>
<td>75 (5)</td>
<td>60 (5)</td>
</tr>
</tbody>
</table>

Table 2. Pharmacokinetic parameters of dolutegravir among 2 doses (dolutegravir 50 mg once daily with food and dolutegravir 50 mg twice daily) in rifampicin treated TB/HIV coinfected patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>QD-BD (n=12)</th>
<th>TLD (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>2.07 (1.30-2.98)</td>
<td>2.46 (2.02-3.12)</td>
<td>0.64 (0.33-1.37)</td>
</tr>
<tr>
<td>AUC0–τ (ng*h/mL)</td>
<td>19.98 (14.04-28.44)</td>
<td>19.37 (14.38-28.57)</td>
<td>1.97 (0.77-4.89)</td>
</tr>
<tr>
<td>Cmin (ng/mL)</td>
<td>0.12 (0.09-0.18)</td>
<td>0.39 (0.23-0.65)</td>
<td>0.01 (0.02-0.02)</td>
</tr>
<tr>
<td>Cmax&gt;0.046 μg/mL</td>
<td>10 (83%)</td>
<td>10 (83%)</td>
<td>-</td>
</tr>
<tr>
<td>Cmax&gt;0.21 μg/mL</td>
<td>10 (83%)</td>
<td>10 (83%)</td>
<td>-</td>
</tr>
<tr>
<td>Cmax&gt;0.23 μg/mL</td>
<td>10 (83%)</td>
<td>10 (83%)</td>
<td>-</td>
</tr>
<tr>
<td>Cmax&gt;0.25 μg/mL</td>
<td>10 (83%)</td>
<td>10 (83%)</td>
<td>-</td>
</tr>
</tbody>
</table>

1Conclusion: The observed data suggest that the relative intracellular disposition of DRV increases with RIF; DTG intra-PBMC/plasma Cmax ratios showed a slight increase (P = 0.068) between DRV/QD and DRV/TLD double doses: median 0.24 (0.21 – 0.25) and 0.29 (0.26 – 0.32), respectively.

1Efficacy and PK of Dolutegravir 50 mg QD with Food Versus 50 mg BID with Rifampicin. 130
372 POTENTIAL DRUG-DRUG INTERACTIONS IN HOSPITALIZED COVID-19 PATIENTS (CATCO-DDI)

Alice Tseng1, Nancy Sheehan2, Kendra Hewlett3, Alison Y. Wong1, Maria Kulikova1, Bryan Coburn1, Rob Fowler1, Matthew P. Cheng2, Srinivas Murthy1
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Background: Therapies for managing COVID-19 disease may interact with other drugs, particularly in hospitalized patients with comorbidities. We characterized the prevalence of potential drug-drug interactions (DDIs) between investigational/approved medications for managing COVID-19 (COVID-meds) and co-medications (co-meds) in hospitalized COVID-19 patients.

Methods: Multicentre retrospective observational study of hospitalized COVID-19 patients screened for the CATCO arm of the SOLIDARITY trial between 1-Apr-20 and 15-Sep-20. Patients’ co-meds at screening were assessed for potential DDIs with the following COVID-meds: hydroxychloroquine (HQ), lopinavir/ritonavir (LPV), remdesivir (REM), dexamethasone (DEX), azithromycin (AZ), interferon beta-1b (INFN) and tocilizumab (TOC). The Liverpool-COVID DDI website and Lexicomp were used to identify and characterize DDI severity (red: most commonly psychotropics, anticoagulants/antiplatelets), while REM and INFN were characterized as significant (red/amber) DDI between each COVID-med and co-med. Secondary outcomes included DDI severity and potential clinical impact and prevalence of DDIs between co-meds. Descriptive statistics are presented as medians (range) or proportions.

Results: Data from 51 patients are available: 61% male, age 74 (44-95) years, number of comorbidities 6 (1-15), Tsidale risk score 6 (31.4% moderate risk, 11.8% high risk) and 10 (0-19) co-meds. LPV had the highest rate of potential DDIs (92.2%, 45% red, 1 DDI per patient) with risk of increased co-med toxicity (most commonly psychotropics, anticoagulants/antiplatelets), while REM and INFN had the least (2% and 9.6%, respectively). Most patients (75%) had ≥1 DEX DDI (primarily amobarbital, 1 per patient) with risk of increased co-med toxicity (Figure 1). The most common DDIs with HQ and AZ involved increased risk of QTc prolongation. Lexicomp identified co-med/co-med DDIs in 62.7% of patients (88% amber, 12% red) most often with increased risk of drug toxicity, commonly involving heparin/LMWH, opioids and antiplatelets. Over one-third (35%) of patients were deemed ineligible for CATCO at screening due to DDIs with LPV.

Conclusion: Hospitalized COVID-19 patients are at high risk of DDIs with many investigational or approved COVID medications. Routine DDI screening is recommended, ideally using both general and COVID-specific DDI resources.
IN SILICO PREDICTION OF LONG-ACTING CABOTEGRAVIR PK IN LIVER-IMPAIRED PATIENTS

Nicolas Cottura1, Maira C. Montanha1, Fazila S. Bunglawala1, Sandra Grañana-Castillo1, Hannah Kinvig1, Marco Siccardi1

1University of Liverpool, Liverpool, UK

Background: Intramuscular (IM) long acting (LA) antiretrovirals can provide pharmacological options to simplify regimens and improve adherence. Currently, two IM LA drugs have been developed, rilpivirine (RPV) and cabotegravir (CBV), however the impact of liver impairment on their pharmacokinetics (PK) has not been fully elucidated. The aim of this project was to predict the PK of IM LA CBV in patients with liver impairment conditions using physiologically-based pharmacokinetic (PBPK) modelling.

Methods: A whole-body IM PBPK model was designed in Simbiology v.5.8.1 (MATLAB R2018b) and used to simulate 100 healthy and liver impaired adults aged 18-60 years. The model was assumed to be qualified if the simulated AUC and Cmax were within 2-fold of the mean reported values, using the absolute average fold error (AAFE) approach as per convention. The model was validated using both oral (30 mg QD) and IM administration (800 mg followed by maintenance dose of 800mg 3 months later) clinical data on CBV. The PBPK model used first order kinetics to describe the IM LA drug release process. Virtual liver impaired patients were classified following the Child-Pugh (CP) score. Equations describing organ and tissue blood flows, plasma protein concentrations and hepatic metabolic enzyme changes were optimised according to each CP score.

Results: The CBV PBPK model successfully passed the validation criteria, as shown in Table 1. Predictions for the IM dose of CBV showed a decrease of 8, 23 and 50% for AUC, Cmax and Ctrough, respectively in CP-A, B and C conditions. A portion of the patients with CP-C liver impairment are predicted to have total plasma Cmax below the PAC90 (660 ng/mL). However, the unbound CBV plasma concentrations are predicted to be comparable to healthy individuals for all patients with liver impairment due to the increase of free drug fraction.

Conclusion: These data suggest that IM LA CBV may be used safely in patients with liver impairment, considering the overall steady-state and increment in unbound plasma concentrations. This approach could also be utilised for patients with liver impairment, considering the overall steady-state and increment in unbound plasma concentrations. This approach could also be utilised for patients with liver impairment.

PHARMACOKINETICS OF LENACAPAVIR, AN HIV-1 CAPSID INHIBITOR, IN HEPATIC IMPAIRMENT

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Background: Lenacapavir (LEN, GS-6207), a potent, selective, first-in-class, multi-stage inhibitor of HIV-1 capsid function is in clinical development as a long acting agent to treat HIV-1 infection, supporting weekly (oral LEN) or less frequent dosing (subcutaneous LEN). In people living with HIV, LEN has shown potent antiviral activity and is well tolerated. This study was conducted to evaluate the effect of moderate hepatic impairment (HI) on the pharmacokinetics (PK) of oral LEN to inform dosing recommendations in patients with mild and moderate HI.

Methods: Participants with moderate HI (Child-Pugh Turcotte [CPT] classification B; score 7-9) and healthy controls (HC) matched for age (±10 years), sex, race and BMI (±15%) received a single oral dose of LEN 300 mg with food (moderate fat meal). Plasma PK was collected through Day 92 post dose; protein binding of LEN was assessed. Preliminary PK parameters were estimated using noncompartmental analysis, and geometric least squares mean (GLSM) ratios and 90% confidence intervals (CI) for AUCinf and Cmax were calculated (GLSM: HI:HC). Safety was evaluated throughout the study.

Results: 20 participants (N=10 with moderate HI and N=10 HC) were enrolled in the study. Preliminary LEN exposure, as assessed by AUCinf and Cmax, was ~1.5-fold and ~2.6-fold higher respectively, in subjects with moderate HI, as compared to HC (Table 1). LEN plasma protein binding, median t1/2 and tmax were similar in both groups. Exploratory analyses indicated no relevant relationships between LEN exposure and CPT score or individual elements of CPT classification. Additionally, no correlation is observed between LEN exposure and subjects’ weight or age. Study treatment was well tolerated. No participant experienced serious or Grade 3 or 4 treatment emergent adverse events.

Conclusion: LEN AUC and Cmax were 1.5- and 2.6-fold higher respectively, in moderately hepatically impaired participants as compared to healthy controls. Based on cumulative safety data in the LEN SC and oral clinical program, no dose adjustment of LEN is recommended in patients with mild to moderate hepatic impairment.

MODEL-INFORMED DOSE SELECTION FOR ISLATRAVIR/MK-8507 ORAL ONCE-WEEKLY PHASE 2B STUDY

Bhargava Kandala1, Craig Fancourt1, Hari Krishna Ananthula1, Youfang Cao1, Pavan Vaddady1, Emmat Asante-Apiah1, Tracy L. Diamond1, Elizabeth G. Rheel1, Randolph P. Matthews1, Wendy Ankrom1, Vamshi Jogiraju1, John Ling1, Polina German1

1Merck & Co, Inc, Kenwaytown, NJ, USA

Background: The novel 2-drug, once weekly (QW) oral combination of Islatravir (ISL) and MK-8507 is in development for the treatment of HIV-1, with the potential to decrease pill burden and dosing frequency. ISL is a first in class NRTTI that is being developed for treatment and prevention of HIV-1. Single doses of MK-8507, a novel NNRTI, achieved robust viral load declines for at least a week post-dose in treatment-naive people living with HIV (PLWH). Dose selection was determined via modeling and simulation for an ISL+MK-8507 dose ranging Phase 2b study. [NCT04564547]

Methods: Concentrations of ISL-triphosphate (ISL-TP), the intracellular active moiety, following QW administration of ISL were predicted using a population pharmacokinetic (PK) model. MK-8507 concentrations were also predicted using a population PK model. A Viral Dynamics Model (VDM) for ISL and MK-8507 was used to predict efficacy for a range of QW doses of ISL (5 – 30 mg) and MK-8507 (50 – 400 mg). The VDM combines PK (drug exposures and the
associated population variability), pharmacodynamic inhibitory effect (clinical IC₅₀ of ISL and MK-8507 estimated from treatment-naïve monotherapy studies; IC₅₀ fold reduction due to resistance-associated variants) and viral dynamics to predict trial outcome as measured by percent efficacy (% of participants with HIV-1 RNA below 50 copies/mL at 48 weeks). A real-world adherence model developed based on a claims database of PLWH receiving Abacavir/Dolutegravir/Lamivudine QD was applied.

Results: A single dose of ISL 20 mg QW achieves ISL-TP trough concentrations comparable to steady state trough levels of ISL-TP for a dose of 0.75 mg QD, a dose shown to provide coverage for wild-type and common NRTI resistance associated variants including M184V. VDM simulations demonstrated that an oral 2-drug QW regimen containing ISL 20 mg in combination with MK-8507 100 mg, 200 mg or 400 mg doses provides 1) at least 90% efficacy and antiviral activity against the most common NRTI and NNRTI resistance-associated variants and 2) robust viral load suppression and efficacy in the event of a late or missed dose and real-world adherence patterns.

Conclusion: This analysis supports selection of ISL 20 mg in combination with MK-8507 100 mg, 200 mg or 400 mg for further development as a QW regimen.

377 IN SILICO PREDICTION OF MONTHLY BICTEGRAVIR MICRONEEDLE ARRAY PAVCHES

Hannah Kinvig¹, Fazila S. Bunglawala¹, Nicolas Goutta¹, Maiara C. Montanha¹, Andrew Lloyd⁴, Kurtis Moffatt¹, Chunyang Zhang⁴, Ryan Donnelly⁴, Marco Siccardi²
¹University of Liverpool, Liverpool, UK, ²Queen’s University Belfast, Belfast, UK

Background: Microneedle array patches (MAPs) comprise of multiple micron-scale needles that can provide painless administration of long-acting nanoformulated drug, producing effective drug plasma concentrations over extended periods of time. The current study used physiologically-based pharmacokinetic (PBPK) modelling to predict optimal dosing strategies of bictegravir (BIC) via MAP administration.

Methods: A whole-body PBPK model was designed in Simbiology v.9.9 (MATLAB R2018a) and used to simulate 100 healthy individuals aged 18-60 years. Transdermal MAP administration was verified previously using cabotegravir and rilpivirine and was implemented into the BIC PBPK model. The BIC model was verified against reported clinical data for the oral administration of 5mg-100mg BIC once daily (QD). The PBPK model was assumed to be verified if the simulated values were within 0.8-1.55-fold of the reported clinical values and if the absolute average-fold error (AAFE) was below 1.5. The verified BIC model was used to simulate three once-monthly (QMT) MAP administrations following four weeks of 50mg BIC QD oral administration as oral lead in. Two dosing strategies were assessed, three MAPs with the same dose and one MAP with a higher loading dose followed by two MAPs with a lower maintenance dose. The protein adjusted (PA)-IC₅₀ of BIC (162 ng/mL) was considered as the minimum target plasma concentration. A range of MAP doses with varying release rates were simulated to determine the most efficient dosing strategy that achieved the target concentration.

Results: The BIC PBPK model was successfully verified according to the criteria. MAP doses between 140-180 mg were simulated for the administration of three identical MAPs, with release rates between 0.0005-0.0025 h⁻¹ being assessed. The optimised dose and release rate from these simulations were then applied as the loading dose with a range of MAP doses between 90-130 mg being simulated for the maintenance dose. The simulated minimum concentration (Cmin) of BIC at the end of each MAP dosing interval for the optimised strategies as the loading dose with a range of MAP doses between 90-130 mg being simulated for the maintenance dose. The protein adjusted (PA)-IC₅₀ of BIC (162 ng/mL) was considered as the minimum target plasma concentration. A range of MAP doses with varying release rates were simulated to determine the most efficient dosing strategy that achieved the target concentration.

Conclusion: The BIC PBPK model was successfully verified across DEX, midazolam and propranolol with an AUC-24 average fold of 1.1 and 0.95; AAFE value of 1.1 and 1.2 for healthy and LD individuals, respectively. When compared to healthy adults, the simulated systemic clearance of DEX decreased and the plasma concentrations increased in all patients with LD, as shown in Table 1. Moreover, a significant difference was observed between the AUCO-24 of DEX PO when comparing no shunting and shunting in patients with CP-B and -C. The increased exposure of DEX in different stages of LD was predicted through PBPK modelling, providing a rational framework to predict PK in complex clinical scenarios related to COVID-19. Although DEX exposure was predicted to be more than 2 times higher in CP-C individuals, no dose adjustments seem necessary in patients with LD considering DEX’s low hepatic extraction, the low dose administered in the COVID-19 protocol and the therapeutic index of DEX.

378 PBPK MODELING OF DEXAMETHASONE IN PATIENTS WITH COVID-19 AND LIVER DYSFUNCTION

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¹University of Liverpool, Liverpool, UK

Background: Patients with pre-existing multimorbidity and liver dysfunction (LD) are more likely to develop severe COVID-19 and have a higher risk of mortality. In severe COVID-19 patients who are mechanically ventilated or require supplemental oxygen, the administration of dexamethasone (DEX) may be life-saving, however the impact of LD on the pharmacokinetics (PK) of DEX is unknown. The aim of the study was to apply PBPK modelling to predict the effect of LD on the PK of DEX in the treatment of COVID-19.

Methods: A whole-body PBPK model was designed in Simbiology v.9.9 (MATLAB R2019a) and used to simulate 100 adult individuals. First the model was qualified against reported clinical data for oral (PO) and intravenous (IV) DEX in healthy adults. Physiological changes and portal vein shunt were incorporated into the model to provide a mathematical description of LD that was classified by Child-Pugh (CP) scores A, B and C. The LD model was qualified against IV and PO reported clinical data for both propranolol (healthy adults and CP-A, -B and -C patients) and midazolam (healthy adult and cirrhotic patients). The model was assumed to be verified if the simulated values were within 2-fold of the reported clinical values and if the absolute average-fold error (AAFE) was below 2. The qualified model was then used to simulate the administration of DEX 6 mg (COVID-19 protocol) in patients with LD (CP-A, -B and -C) with and without shunting. The mean shunt index (%) considered in the model was 40 ± 18.

Results: The PBPK model was successfully qualified across DEX, midazolam and propranolol with an AUC-24 average fold of 1.1 and 0.95; AAFE value of 1.1 and 1.2 for healthy and LD individuals, respectively. When compared to healthy adults, the simulated systemic clearance of DEX decreased and the plasma concentrations increased in all patients with LD, as shown in Table 1. Moreover, a significant difference was observed between the AUCO-24 of DEX PO when comparing no shunting and shunting in patients with CP-B and -C. The increased exposure of DEX in different stages of LD was predicted through PBPK modelling, providing a rational framework to predict PK in complex clinical scenarios related to COVID-19. Although DEX exposure was predicted to be more than 2 times higher in CP-C individuals, no dose adjustments seem necessary in patients with LD considering DEX’s low hepatic extraction, the low dose administered in the COVID-19 protocol and the therapeutic index of DEX.

379 IN VIVO EVALUATION OF LONG-ACTING BIODEGRADABLE EMTRICITABINE IMPLANTS

Megan Neary¹, Joanne Sharp¹, Paul Curley¹, Henry Pertinez¹, Helen Box¹, Lee Tatham¹, Danielle Brain¹, Fayeh Hemi¹, Anika Shaikh¹, Chung Liu¹, Careen Meyers¹, Charles W. Flexner¹, Steve Rannard², Andrew Owen¹
¹University of Liverpool, Liverpool, UK, ²The Johns Hopkins University, Baltimore, MD, USA

Background: Long-acting (LA) antiretroviral interventions hold promise to revolutionise HIV therapy and prevention and mitigate key concerns regarding adherence to oral medications. The current study investigated a biodegradable subcutaneous implant derived from a polymer formed entirely from an entecitabine (FTC) prodrug (POP implant).

Methods: For in vitro studies, POP implants were incubated in 1 mL of phosphate buffered saline containing human liver microsomes at 37 °C/ 125 rpm for 72 hours. Every 24 hours, 500 µL samples were taken and replaced with 500 µL of fresh buffer to maintain sink conditions. For in vivo evaluations, two POP implants (2mm x 15mm each) were inserted subcutaneously into the scapular region of male Wistar rats under anaesthetic with 3% isoflurane.

Table 3. Simulated pharmacokinetic parameters of Emtricitabine following oral and subcutaneous administration in Child-Pugh A, B and C patients with and without shunting.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CP-A</th>
<th>CP-B</th>
<th>CP-C</th>
<th>CP-A</th>
<th>CP-B</th>
<th>CP-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng.h/mL)</td>
<td>850-1800</td>
<td>850-1800</td>
<td>850-1800</td>
<td>850-1800</td>
<td>850-1800</td>
<td>850-1800</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>50-150</td>
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<td>50-150</td>
<td>50-150</td>
<td>50-150</td>
<td>50-150</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>0.7-1.5</td>
<td>0.7-1.5</td>
<td>0.7-1.5</td>
<td>0.7-1.5</td>
<td>0.7-1.5</td>
<td>0.7-1.5</td>
</tr>
</tbody>
</table>

The PBPK model was successfully qualified across DEX, midazolam and propranolol with an AUC-24 average fold of 1.1 and 0.95; AAFE value of 1.1 and 1.2 for healthy and LD individuals, respectively. When compared to healthy adults, the simulated systemic clearance of DEX decreased and the plasma concentrations increased in all patients with LD, as shown in Table 1. Moreover, a significant difference was observed between the AUCO-24 of DEX PO when comparing no shunting and shunting in patients with CP-B and -C. The increased exposure of DEX in different stages of LD was predicted through PBPK modelling, providing a rational framework to predict PK in complex clinical scenarios related to COVID-19. Although DEX exposure was predicted to be more than 2 times higher in CP-C individuals, no dose adjustments seem necessary in patients with LD considering DEX’s low hepatic extraction, the low dose administered in the COVID-19 protocol and the therapeutic index of DEX.
Plasma samples were obtained 1-6 hours and 1-31 days post implantation. FTC concentrations were quantified using a validated LC-MS/MS assay.

**Results:** Eleven candidates were screened in vitro and three were selected for animal studies with in vitro release rates of 145, 59 and 7 μg/day for POP1, POP2 and POP3, respectively. In vitro release rate was determined from the linear phase of the profile. The plasma pharmacokinetics of the three implants are shown in Figure 1, with a Cmax of 5581, 2306 and 1847 ng/mL, AUC0-tlast of 14,614, 4644, and 4616 ng·days/mL and Cmin of 10, 27 and 32 ng/mL for POP1, POP2 and POP3, respectively. Plasma concentration profiles following implantation could be empirically described by a 2-compartment model with first order input, reflecting the drug release and flip-flop kinetics rather than the intrinsic pharmacokinetic disposition of FTC seen previously for intravenous administration. Apparent clearance was 0.7, 1.6 and 2.0 L/h/kg with final phase half-life of 6.6, 17.3, and 14.6 days for POP1, POP2 and POP3 respectively. In vitro-in vivo correlation for AUC0-tlast and Cmax revealed R2 values of 0.87 and 0.93, and an inverse correlation with Cmin (R2 = 0.98), demonstrating the relationship between in vitro release and in vivo exposure.

**Conclusion:** These data support LA drug delivery from a biodegradable polymer implant manufactured exclusively from an FTC prodrug for over 31-days. The relevance of human target exposures in rat studies are unclear, but concentrations remained above the reported FTC EC50 for 14, 10 and 7 days for POP1, POP2 and POP3, respectively. Ongoing studies seek to optimise exposures that can be achieved for FTC and POP implants manufactured from other antiretroviral drugs.

**Results:** Ninety-six patients completed panel testing with 656 clinically relevant pharmacogenomic results (109 major, 547 mild-moderate). Eighty-nine patients completed follow-up visits with the HIV pharmacist, and 47 (53%) were provided new clinical recommendations based on their current medication profile, the majority related to monitoring for efficacy or toxicity. Clinical recommendations were associated with a major genetic finding or determined to be of significant clinical importance in twenty patients (22%). Panel results offered potential explanations of prior ART intolerance in 38% of patients and explanation for ART inefficacy in 1 patient. Genetic explanation for non-ART toxicity was seen in 20% of patients, with genetic contributors to inefficacy of non-ART therapy identified in 37% of patients.

**Conclusion:** Preliminary data in a small cohort of PLWHIV demonstrates benefit of routine pharmacogenomic panel testing.

### Table: Clinical findings by patient after pharmacogenomic panel result review

<table>
<thead>
<tr>
<th>ART</th>
<th>Other medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>35 (39%)</td>
</tr>
<tr>
<td>Adjustment</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Monitoring</td>
<td>17 (19%)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**Background:** Pharmacogenomics is the area of individualized medicine focused on genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and drug targets that explain inter-individual variation in drug efficacy and toxicity. Single gene assessment of HLA-B*57:01 prior to abacavir has become economically feasible and scalable to larger populations, providing more detailed information to enhance safe and effective medication therapy for PLWHIV. Clinical data on the utility of pharmacogenomic panels in PLWHIV is not available. Our study aimed to assess the impact of pharmacogenomic panel testing in PLWHIV.

**Methods:** One hundred PLWHIV were provided a comprehensive pharmacogenomic panel during routine care visits within the HIV specialty clinic of a large academic medical center. The panel determined the presence of specific genetic variants that could predict response or toxicity to commonly prescribed antiretroviral therapy (ART) and non-ART medications. An HIV specialty pharmacist reviewed the results with patients and the care team. The pharmacist: (1) recommended clinically actionable interventions based on the patients current drug therapy, (2) assessed for genetic explanations for prior medication failures, adverse effects, or intolerances, and (3) advised on potential future clinically actionable care interventions based on individual genetic phenotypes.

**Background:** SARS-CoV-2 is a single-stranded positive-sense RNA virus that utilizes a negative-sense subgenomic (sg)RNA intermediates for viral protein synthesis. We developed a synthetic RNA (“hijack RNA”) that is designed to be recognized by SARS-CoV-2 RNA-dependent RNA polymerase (RdRpn). Upon recognition, hijack RNA is transcribed into diphtheria toxin fragment A (DT-A), to induce death specifically in infected cells, which could be a potential treatment (Fig 1A).

**Methods:** Adeno-associated virus (AAV) was packaged with a novel vector expressing our SARS-CoV-2 hijack RNA, which contains reverse complementary strand of DT-A cDNA, flanked between secondary structures of SARS-CoV-2 sgRNA. Vero and HepG2 cells that were uninfected or infected with SARS-CoV-2 USA-WAI2020 strain at 0.1 MOI, were transduced with test or GFP (control) AAVs. Uninfected jurkat, HEK and BHK-21 cells were also transduced with test AAV to assess off-target effects of hijack RNA. Cell death and viability were evaluated daily by FACS and automated cell count. The same experiments were repeated on SARS-CoV-2 RdRp expressing Vero and HepG2 cells to validate hijack RNA’s specificity to RdRp. SCID mice were subcutaneously injected with HepG2-SARS-CoV-2-Fluc cells to establish an in vivo bioluminescent SARS-CoV-2 infection model. Mice were treated with test AAV two weeks after xenotransplantation. Infected cell killing was monitored by in vivo imaging on IVIS.

**Results:** SARS-CoV-2 infection was eradicated from Vero, Calu3 and HepG2 cultures within 48h after test AAV transduction, confirmed by FACS analysis, cell proliferation assays and the absence of CPE in cell imagery (Fig 1B). Test AAV, or presence of hijack RNA, had no effect on uninfected cells (Fig 1C). Similar results were observed in RdRp expressing cell lines, confirming the hypothesized mechanism of action and the hijack RNA’s dependence on SARS-CoV-2 RdRp. Results of ongoing in vivo studies will be presented.

**Conclusion:** An mRNA delivered or expressed in trans to engage with SARS-CoV-2 RdRp successfully hijacked the virus machinery to induce rapid death in infected cells but not in uninfected cells, resulting in total eradication of the virus within 48h. Hijack RNA’s transcription into the killer molecule DT-A was dependent on viral RdRp, confirming the specificity this potential treatment. This novel approach could be used to develop an effective treatment, potentially in the form of an AAV or an aerosolized RNA drug to rapidly eradicate COVID-19 infection.
the coronavirus disease 2019 (COVID-19). The high morbidity and mortality associated with COVID-19 and the lack of an approved drug or vaccine for SARS-CoV-2 underscores the urgent need for developing effective antiviral therapies. Therapeutics that target essential viral proteins are effective at controlling virus replication and spread. Coronavirus Spike glycoproteins mediate viral entry and fusion with the host cell, and thus, are essential for viral replication. To enter host cells, the Spike proteins of SARS-CoV-2 and related coronaviruses, SARS-CoV, bind the host angiotensin-converting enzyme 2 (ACE2) receptor through their receptor binding domains (RBDS).

**Methods:** We performed comparative analyses of the SARS-CoV and SARS-CoV-2 RBDS-ACE2 interaction interfaces to rationally design a panel of Spike-targeting ACE2-derived peptides (SAPs). Antiviral potencies of SAPs were evaluated against lentiviral vectors pseudotyped with SARS-CoV-2 or SARS-CoV Spike glycoproteins. Affinity precipitation assays were employed to determine the binding affinities of SAPs to recombinant SARS-CoV-2 RBBD. Antiviral potential of selected SAPs was also validated against two pathogenic human coronaviruses, SARS-CoV-2 and HCoV-NL63, both of which use ACE2 as entry receptors.

**Results:** We designed six SAPs – four derived from α1, one derived from α3, and one derived from α11 helix of ACE2. Three of the six SAPs inhibited both SARS-CoV-2 and SARS-CoV Spike-mediated virus infection with IC50 values in the low micromolar range. In vitro, SAP-RBD binding affinities tracked closely with their antiviral IC50 values. Importantly, two SAPs inhibited SARS-CoV-2 and HCoV-NL63 infections. Results from the infection experiments and modeling of the peptides with RBBD identified a six amino acid (Glu37-Gln42) ACE2 motif that is important for SARS-CoV-2 inhibition.

**Conclusion:** We rationally designed a panel of ACE2-derived peptides based on the RBBD-ACE2 binding interfaces of SARS-CoV-2 and SARS-CoV. We identified two peptides that inhibited infection with genuine SARS-CoV-2. Our work demonstrates the feasibility of inhibiting SARS-CoV-2 with peptide-based inhibitors. These findings will allow for the successful development of engineered peptides and peptidomimetic-based compounds for the treatment of COVID-19.

**RNHC INHIBITS SARS-CoV-2 IN VITRO BUT IS MUTAGENIC IN MAMMALIAN CELLS**

**Shuntai Zhou**, Collin Hill, Sanjay Sarkar, Victor Tse, Timothy Sheahan, Ralph Baric, Mark Heise, Ronald Swanstrom

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**Background:** We previously showed that β-D-N4-hydroxyctydine (RNHC) and its orally bioavailable prodrug, molnupiravir, acts as a broad-spectrum antiviral against coronaviruses in vitro and in vivo through lethal mutagenesis. Molnupiravir is currently in clinical trials for the treatment of SARS-CoV-2 infection. However, there are concerns that RNHC could be metabolized to dihydorRNHC and cause mutations in host cells. We examined the in vitro antiviral and mammalian cell mutagenic activity of three different nucleoside/base analogs, RNHC, favipiravir, and ribavirin, on SARS-CoV-2. We further examined the in vitro genotoxicity of a panel of antiviral nucleotide/nucleoside analogs, including RNHC, using a modified HPRT gene mutation assay.

**Results:** We designed six SAPs – four derived from α1, one derived from α3, and one derived from α11 helix of ACE2. Three of the six SAPs inhibited both SARS-CoV-2 and SARS-CoV Spike glycoproteins. Antifusion potencies of SAPs were evaluated against lentiviral vectors pseudotyped with SARS-CoV-2 or SARS-CoV Spike glycoproteins. Affinity precipitation assays were employed to determine the binding affinities of SAPs to recombinant SARS-CoV-2 RBBD. Antiviral potential of selected SAPs was also validated against two pathogenic human coronaviruses, SARS-CoV-2 and HCoV-NL63, both of which use ACE2 as entry receptors.

**Conclusion:** We rationally designed a panel of ACE2-derived peptides based on the RBBD-ACE2 binding interfaces of SARS-CoV-2 and SARS-CoV. We identified two peptides that inhibited infection with genuine SARS-CoV-2. Our work demonstrates the feasibility of inhibiting SARS-CoV-2 with peptide-based inhibitors. These findings will allow for the successful development of engineered peptides and peptidomimetic-based compounds for the treatment of COVID-19.

** RNHC INHIBITS SARS-CoV-2 IN VITRO BUT IS MUTAGENIC IN MAMMALIAN CELLS**

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1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

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potential as an orally bioavailable direct acting antiviral against SARS-CoV2 early in infection, especially in high risk patients. However, clinical use should be carefully considered in light of its potential mutagenic effects on the host.

(a) 

(b) Mismatch compared to reference HPRT mRNA sequence

![Fig. 1: HPRT assay to detect genotoxicity of β-D-N4-hydroxycytidine (NHCl), ribavirin (RBV), favipiravir (FAV), ribozidovine (AZT) and tenofovir (TDF) in CHO-K1 cells in vitro.](image)

**Methods:** In the HPRT EXP2, an additional round of cloning for spontaneous HPRT mutation was conducted to limit the background mutation. Each compound/dose group had 3 replicates. Average numbers of colonies are shown on the top of each bar. (b) The highlighter plot of the HPRT mutation colony scoring from the second experiment. HPRT colonies were scored from the cell culture dishes and transferred into 24-well tissue culture plates in complete growth medium with 3μM 6-TG for 4 days. Cells in each well were collected and total RNA was extracted. We amplified the HPRT mRNA using one-step RT-PCR and sequenced the PCR products with Sanger sequencing The total sequenced region was 883 bases of the total 1179-base HPRT mRNA (NM_000164.5:2), with regions on each end of the mRNA not covered by sequencing. Each colony sequence was compared with the reference mRNA sequence. A total of 42 colonies were sequenced and 32 of them had base substitutions or frame-shifts from deletions. Most of the mutations are different, while a few colonies contained the identical mutation. Notably, we found 3 colonies in one NHC 3μM replicate and 2 colonies from another NHC 3μM replicate had identical mutations.

**385 ANTI–SARS-CoV-2 MULTI-DOMAIN DARPin® MOLECULES AS HIGHLY POTENT THERAPEUTICS**


Molecular Partners AG, Zürich-Schlieren, Switzerland; *Spiez Laboratory, Spiez, Switzerland; Utrecht University, Utrecht, Netherlands; Integrative Biologics, Basel, Switzerland; Vinoolclins Xplore, Schaff, Netherlands

**Background:** Globally accessible preventive and therapeutic drugs against SARS-CoV-2 are urgently needed. Here, we report the generation and characterization of the first anti-SARS-CoV-2 DARPin® molecules with therapeutic potential. DARPin® molecules are an emerging class of novel therapeutics based on naturally occurring ankyrin repeat motifs which can be rapidly produced in bacteria in large quantities.

**Methods:** From a naive library of 1012 DARPin molecules 380 molecules were selected to target the SARS-CoV-2 spike protein. Extensive biophysical and biochemical characterization, pseudovirus and infectious virus neutralization assays as well as cryo-EM analysis, resulted in 11 highly distinct single domain DARPin molecules which were used for the assembly of highly potent multi-domain DARPin molecules. The protective efficacy of multi-domain DARPin molecules was studied in COVID-19 hamster models.

**Results:** From the 11 single domain DARPin molecules a range of multi-domain DARPin molecules were assembled which were grouped into multi-paratopic DARPin molecules neutralizing the receptor binding domain (RBD) and multi-mode DARPin molecules targeting simultaneously the RBD, the S1 N-terminal-domain (NTD) and/or the S2 domain. Multi-domain DARPin molecules binding three spike protein domains simultaneously demonstrated increased binding affinity, virus neutralization potency and the potential to prevent viral escape via mutations. Cryo-EM analysis further supported the structural understanding of the multi-domain DARPin molecules and molecular modelling proved that simultaneous binding of individual DARPin domains to various spike protein domains is feasible. Two additional DARPin domains binding human serum albumin were incorporated in the DARPin molecules, conferring an expected half-life of about 3 weeks in humans. A multi-paratopic RBD-neutralizing DARPin molecule and a multi-mode DARPin molecule were found to potently block SARS-CoV-2 infection with IC50 values in the single-digit ng/mL range. Multi-paratopic DARPin molecules proved prophylactic and therapeutic efficacy in hamster SARS-CoV-2 infection models.

**Conclusion:** The anti-SARS-CoV-2 multi-domain DARPin molecule, ensovibep or MP0420, which entered clinical phase I in November 2020, displayed very high antiviral potency, rapid and high production capacity due to bacterial fermentation and demonstrated prophylactic and therapeutic activity in hamster SARS-CoV-2 infection models.

**IDENTIFICATION OF REPURPOSING DRUGS AGAINST SARS-CoV-2 USING HUMAN LUNG TISSUES**

Judith Grau-Expósito, David Pereira, Nuria Massana, Marina Suppi, Joel Rosado, Javier García-Pérez, José Alcamí, Anna Serrano, Vicenç Falcó, Meritxell Genesca, María José Buzón

Vall d’Hebron Research Institute, Barcelona, Spain, Hospital Universitario de la Vall d’Hebron, Barcelona, Spain; Institute de Salud Carlos III, Majadahonda, Spain; Hospital Clinic of Barcelona, Barcelona, Spain

**Background:** No effective drugs against SARS-CoV-2 infection are available. Screening of therapeutic candidates is primarily performed using immortalized cell lines. However, primary cell targets might show intrinsic differences in the expression profile of relevant host proteins, required for viral replication that could significantly affect the activity and potency of antivirals. Thus, the development of more physiological models for antiviral drug screening are urgently needed.

**Methods:** Lung tissue was obtained from routinely thoracic surgical resections and was immediately digested before experiment set up. Cell populations and expression of ACE2 were characterized by FACS, and cell targets for SARS-CoV-2 were identified using a VSV*ΔG(GFP)-S pseudotyped virus. 39 repurposing drugs previously identified by in silico models as potential viral entry inhibitors were tested using a VSV*ΔG(Glu)-S virus. Cytopathic concentration (CC50) and inhibitory concentration (IC50) values were calculated using a non-linear regression dose-response curve and were compared to drug activity in VeroE6 cells.

**Results:** Alveolar type II (AT-II) cells, the main cell target for SARS-CoV-2 infection in lungs, were identified within a fraction of cells characterized by CD45-, CD31-, EpCAM+ and HLA-DR+, (~0.01-0.5% of viable cells). Using an anti-ACE2 antibody (Figure 1). Despite low and variable numbers of AT-II targets, antiviral assays using VSV*ΔG(GFP)-S were highly sensitive and reproducible (CV of 17%). Compared with VeroE6 cells, IC50 values tended to be higher in tissues. Moreover, we found that 12.8% of the tested compounds had discordant results, where 10.25% of the drugs showed some antiviral effect in lungs but no activity in VeroE6 and 3.9% showed only antiviral effect in VeroE6. Modulation of ACE2 expression by some of these compounds was also highly discordant between the cell line and lung tissue. Cepharantin (IC50=6μM, CC50=14μM) and Erogolid (IC50=4.3μM, CC50=24μM) were identified as the most active entry inhibitors in lung cell suspension.

**Conclusion:** The use of lung tissue for the screening of antiviral compounds represents a valid physiological and relevant model, which evidences intrinsic
discrepancies with cell lines. Importantly, we identified repurposing drugs against SARS-CoV-2 with potential for clinical testing.

**387 THE 3CLpro INHIBITOR ALG-097111 POTENTLY INHIBITS SARS-CoV-2 REPLICATION IN HAMSTERS**

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**Background:** There is an urgent need for potent drugs for the treatment or prevention of SARS-CoV-2 infections. Inhibition of viral proteases has been proven a successful therapeutic strategy for infections with HIV and HCV. Most reported inhibitors of the SARS-CoV-2 3-chymotrypsin-like (3CL) cysteine protease also target cathepsin L; the latter is involved in the SARS-CoV-2 entry process. We aim to develop potent and selective 3CL protease inhibitors devoid of cathepsin L inhibition.

**Methods:** Structure-based optimization and biochemical profiling, resulted in ALG-097111, a potent and selective SARS-CoV-2 3CL protease inhibitor. ALG-097111 was profiled in vitro in SARS-CoV-2 and CoV-OC43 cellular assays. In vitro microsomal stability and in vivo PK evaluation in rodents, in presence of the CYP-inhibitor ritonavir, was followed by the evaluation of ALG-097111 in a SARS-CoV-2 infection model in hamsters.

**Results:** ALG-097111 exhibits potent SARS-CoV-2 3CLpro activity (IC50 = 0.007 µM) with no associated cathepsin L inhibition (IC50 > 10 µM). This selectivity extended to other human proteases, displaying less than 50% inhibition at 10 µM, as well as receptor and kinase panels. While ALG-097111 is stable in human and dog microsomes (t1/2 > 60 min) and hepatocytes (t1/2 > 360 min), ALG-097111 showed lower stability in hamsters specifically (t1/2 = 15 min). Addition of ritonavir to the hamster microsome assay increased the in vitro half-life (t1/2 = >60 min). When administered subcutaneously with oral co-dosing of ritonavir, ALG-097111 shows high plasma and lung exposures. Dosing hamsters with ALG-097111, followed by intranasal SARS-CoV-2 infection led to a significant prevention of SARS-CoV-2 infections. Inhibition of viral proteases has been proven a successful therapeutic strategy for infections with HIV and HCV. Most reported inhibitors of the SARS-CoV-2 3-chymotrypsin-like (3CL) cysteine protease also target cathepsin L; the latter is involved in the SARS-CoV-2 entry process. We aim to develop potent and selective 3CL protease inhibitors devoid of cathepsin L inhibition.

**Conclusion:** The potency of the ACE2 Decoy (T27Y/H34A) target and ALG-097111, a potent and selective inhibitor of SARS-CoV-2 3CLpro, shows high plasma and lung exposures. Dosing hamsters with ALG-097111, followed by intranasal SARS-CoV-2 infection led to a significant prevention of SARS-CoV-2 infections.

**389 TARGETING THE RECEPTOR AXL BY BEMCENTINIB PREVENTS SARS-CoV-2 INFECTION**

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**Background:** SARS-CoV-2 enters host cells via an interaction between viral spike protein and cellular AXL. However, in common with other enveloped viruses, apoptotic mimicry may also assist cell entry: phosphatidylserine (PS) exposed on the viral envelope interacts with cellular PS receptors leading to efferocytosis. The PS receptor, GA56 bound to AXL, has also been shown to suppress type I interferon (IFN) responses. AXL is the predominant cellular PS receptor expressed on airway-derived cell lines. We hypothesized that the clinical-stage, AXL kinase-specific inhibitor, bemcentinib, inhibits SARS-CoV-2 infection and represents a potential therapy for COVID-19.

**Methods:** Viral infection and host transcriptional responses to infection with SARS-CoV-2 or a VSV pseudovirion bearing SARS-CoV-2 spike were measured in human airway epithelial cell lines, engineered HACE2-expressing A549 lung cancer cells, and Vero E6 cells treated with bemcentinib or protease inhibitors. Studies also measured the effect of bemcentinib on SARS-CoV-2 binding and internalization into cells. In the in vivo effect of bemcentinib (50mg/kg, orally, twice daily) was assessed in C57BL6/J mice infected with the murine coronavirus, mouse hepatitis virus (MHV, 500 or 5x104 infectious units intraperitoneally). Viral titers and loads in liver were evaluated at day 5 post-infection. Spleen and liver were harvested to evaluate type 1 IFN-related gene expression changes.

**Results:** Bemcentinib prevented infection by SARS-CoV-2 as assessed by viral transcripts in RNAseq studies as well as viral load in qRT-PCR analysis of human lung epithelial, A549-HACE2 and Vero E6 cells. Bemcentinib reduced virus internalization without affecting virus binding. Further, bemcentinib inhibition correlated well with inhibitors that block endosomal acidification and cathepsin activity, consistent with AXL-mediated SARS-CoV-2 uptake into endosomes. In vivo, bemcentinib significantly inhibited murine MHV liver titers and virus load and significantly enhanced signatures of type 1 IFN response.

**Conclusion:** The orally bioavailable AXL inhibitor bemcentinib demonstrated potent antiviral effects in pre-clinical SARS-CoV-2 and other coronavirus models. These data support two ongoing phase 2 studies (EudraCT 2020-001736-95 [UK] & CTI/2020/10/028602 [India] and DOH-29-092020-6170 [South Africa])
of bempencitinib for the treatment of COVID-19 in hospitalized patients, including those requiring supplemental oxygen and/or non-invasive ventilation, but not intubation.

**390 MULTICENTER, OPEN-LABELED EFFICACY STUDY OF AVIFAVIR IN PATIENTS WITH COVID-19**

**Suzana Corritori**, Elena Yakubova, Andrey Ivashchenko, Tagir Sidelkov, Alina Ergonova, Elena Merkulova, Andrew Binovec, Nikita Lomakin, Elena Smolyarchuk, Natalia Papazova, Dmitry Kravencho, Sergey Baranovskyy, Jenny Remeeva, Nikolay Savchuk, Alexandre Ivachchenko

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**Background:** AVIFAVIR (famciclovir 200mg tablet formulation) is the first effective direct antiviral drug approved for treatment of mild to severe COVID-19 patients. It is registered in Russia and conditionally approved in several Latin American and Asian countries. This study is a multicenter, open-labeled, comparative clinical study to assess efficacy of Avifavir in patients with COVID-19. The data represent results of post-registration real life practice of AVIFAVIR compared with the Standard-of-Supportive-Care. The analysis included data on 940 patients, of which 470 patients were included in each of the treatment groups.

**Methods:** The average age of patients was 54.9±17.5 and 55.5±19.3 years in AVIFAVIR and SOC groups respectively. 246 (52.3%) patients in AVIFAVIR group and 265 (56.4%) patients in SOC group were female. The average duration of the disease from the onset of the first symptoms to the start of therapy was 4.9±2.2 days in the AVIFAVIR group and 4.7±2.8 days in the SOC group. The average value of saturation index (SpO2, %) in AVIFAVIR group was 94.5±4.8 and in SOC group 94.3±6.6.

**Results:** Evaluation of the efficacy of AVIFAVIR was carried out in comparison with SOC for the following indicators in patients hospitalized with COVID-19: time to virus elimination; time of the improvement of clinical condition to satisfactory, time to normalization of clinical signs (SpO2) and the assessment of the number of responses to therapy. AVIFAVIR showed statistically significant results compared to SOC in terms of faster virological response; more rapid improvement of the clinical condition and higher response rates to therapy. The median time to virus elimination in the AVIFAVIR group was 6 days, while 12 days in the SOC group (which is a statistically significant difference, p<0.001). The median time to normalization of saturation (≥95%) in the AVIFAVIR group was 2 days, vs 4 days in the SOC group (p=0.001). The time median to clinical improvement was 8 days in the AVIFAVIR group and 15 days in the SOC group (p<0.001). The number of responses to therapy in the AVIFAVIR group was statistically significantly higher than in the SOC group after 5 days (p=0.0248) and after 14 days (p<0.001).

**Conclusion:** The study demonstrated the high clinical efficacy and tolerability of an early antiviral therapy with Avifavir. The results show with statistical significance that an early initiation of antiviral therapy with Avifavir is one of major factors in successful treatment and cure of COVID-19 patients.

**391 CASE SERIES: TREATMENT OF COVID-19 WITH CONVALESCENT PLASMA IN B-CELL DEPLETION**

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**Background:** Anti-CD20 therapy is used to treat autoimmune and hematological diseases. An absent or delayed antibody response against SARS-CoV-2 puts patients at risk for a protracted and severe disease course. These patients may benefit from antibody-based therapy of which convalescent plasma (ConvP) is the most broadly available source.

**Methods:** ConvP from donors with SARS-CoV-2 antibody titers was used when their plaque reduction neutralization test (PRNT50) showed a PRNT50 titer of at least 1:320. Patients were successfully treated with only 300ml ConvP.

**Results:** 22 B-cell depleted patients admitted with COVID-19 were treated with ConvP. B-cell depletion was the result of rituximab (n=11), obinutuzumab (n=1), XLA (n=1) or Blinatumab (n=1) for lymphoma, auto-immune disease or ALL. Patients had been sick for a median of 26 days (IQR 18 – 34.5 days) and all were SARS-CoV-2 RBD antibody negative on the day of transfusion. The plasma units had a median PRNT50 titer of 1:640 (IQR 1:160 – 1:1280). All patients showed obvious clinical improvement after the first transfusion. All patients also showed pulmonary improved on a chest CT-scan. All patients seroconverted with a median PRNT50 24 hours after transfusion of 1:40 (Range 1:20 – 1:80) and a median positive Wntalti total Ig OD ratio of 12.63 (range 3.55 – 18.39) (Figure 1). PCR became negative in all patients within 16 days after transfusion and isolation could be lifted at that time.

**Conclusion:** We observed prompt clinical and virological recovery after therapy with ConvP of B-cell depleted patients with a very protracted COVID-19 disease course. Our observation provide a proof of concept that in carefully selected patients, antibody-based therapy can be very effective. 24 hours after the transfusion of 600ml of ConvP, all patients had seroconverted to a PRNT50 titer of 1:20 to 1:80. We therefore suggest an initial dose of 600ml of ConvP with a PRNT50 of at least 1:320.
Conclusion: Prompt clinical and virological recovery after ConvP transfusion was observed in the large majority of B-cell depleted antibody negative patients admitted with COVID-19. Our observation shows that for carefully selected patients, antibody-based therapy can be effective. After transfusion of 600mL of ConvP, all patients had seroconverted to high anti-RBD antibody titers and detectable PRNT50 titers of 1:20 or higher. Based on these observations, we suggest an initial dose of 600mL of ConvP.

393 REMDESIVIR VERSUS STANDARD OF CARE FOR SEVERE COVID-19

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Background: Remdesivir (RDV), a direct-acting nucleotide pro-drug inhibitor of viral RNA-dependent RNA polymerases, was approved by the FDA for the treatment of hospitalized patients (pts) with COVID-19 infection and has been shown to shorten time to recovery and improve clinical outcomes in randomized clinical trials. We present the final Day 28 (D28) analysis of RDV vs standard of care (SOC) (interim Day 14 [D14] analysis published [Olender et al. Clin Infect Dis 2020]).

Methods: Final comparative analysis from two studies: a prospective phase 3, randomized study of RDV (RDV cohort) and a real-world retrospective cohort study of SOC (non-RDV cohort). Both studies enrolled pts with SARS-CoV-2 infection confirmed by polymerase chain reaction, who had oxygen saturation ≤94% on room air or required supplemental oxygen and had pulmonary infiltrates. Pts in the RDV cohort were randomized 1:1 to receive IV RDV for 5 or 10 days (200 mg on Day 1 followed by 100 mg/day on Days 2–5 or 2–10), plus SOC; the two randomized dose–groups were combined for analysis. Pts in the non-RDV cohort received SOC as determined by local treatment practices (excluding RDV). Analysis populations were balanced using propensity score testing cobicistat-boosted darunavir on SARS-CoV-2 patients. As the booster activity of the main protease of SARS-CoV-2 (3CLpro) was measured by FRET assay.

Results: After PS matching, baseline characteristics were generally similar in the RDV and non-RDV cohorts; median age 61 years, 63% male, 42% obese, 12% Black, 71% no/low-flow oxygen use, 25% high-flow oxygen, 3% ventilated. Pts in the RDV cohort had significantly higher D14 clinical recovery rates (65% vs 57%) and significantly lower D28 mortality rates (12% vs 16%) compared with the non-RDV cohort (Table). In the multivariable analysis, in addition to RDV use, a lower risk of death at D28 was associated with: younger age; being female; being White (versus being Black/African American); receiving an HIV protease inhibitor prior to baseline; not having cardiovascular disease or COPD; more days of symptoms prior to baseline; and being on room air or low-flow oxygen at baseline (versus being on invasive mechanical ventilation).

Conclusion: RDV was associated with significantly higher rates of clinical recovery at Day 14 and lower Day 28 mortality compared with SOC in hospitalized pts with severe SARS-CoV-2 infection.

Table: Clinical recovery and mortality rates in hospitalized patients with severe SARS-CoV-2 infection (based on PS matching)

<table>
<thead>
<tr>
<th></th>
<th>RDV cohort N = 368</th>
<th>Non-RDV cohort N = 1399</th>
<th>Odds ratio [95% confidence interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14 clinical recovery, n (%)</td>
<td>249/368 (68.2)</td>
<td>716/1399 (51.7)</td>
<td>1.49 [1.16–1.90]</td>
</tr>
<tr>
<td>Day 28 mortality, n (%)</td>
<td>44/368 (12.2)</td>
<td>226/1399 (16.2)</td>
<td>0.67 [0.47–0.95]</td>
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394 COBICISTAT SYNERGIZES WITH REMDESEVIR TO SUPPRESS SARS-CoV-2 REPLICATION IN VITRO

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Background: The ongoing SARS-CoV-2 pandemic poses an urgent need to identify novel drug treatments that are effective, well tolerated and quickly translatable to a clinical setting.

Methods: In-silico binding modes were predicted by molecular docking. The Bvpat.020 SARS-CoV-2 isolate was used to infect Calu-3, T84, Vero E6 cells and a primary colon organoid at MOIs of 0.05 or 0.5. Supersantam and intracellular SARS-CoV-2 RNA was quantified by RT-qPCR or immunofluorescence (IF). The activity of the main protease of SARS-CoV-2 (3CLpro) was measured by FRET assay. Viral protein expression was assessed by western blot. Syncytia formation was determined by IF in cells expressing the spike protein. Cell viability was determined by MTT and crystal violet staining. Synergism scores were calculated using the SynergyFinder web tool.

Results: In-silico docking using a library of FDA-approved drugs highlighted cobicistat as candidate inhibitor of SARS-CoV-2 3CLpro. Experiments using two different viral MOIs in three different cell lines proved that cobicistat inhibits SARS-CoV-2 replication at non-toxic, low micromolar concentrations (IC50 0.6-9µM; CC50 39-52 µM) (Fig 1A). However, cobicistat did not inhibit 3CLpro activity in FRET assay, while western blot analysis suggested that cobicistat impacts on spike glycoprotein levels/processing. Accordingly, cobicistat decreased syncytia formation in spike-expressing Vero E6 cells. The range of in-vitro antiviral concentrations of cobicistat was compatible with plasma levels reachable in mice and humans, but above those achieved through standard dosages used to boost HIV-1 protease inhibitors, in line with the failure of trials testing cobicistat-boosted darunavir on SARS-CoV-2 patients. As the booster activity of cobicistat is exerted through inhibition of Ecto-CTPase M50A (CYP3A) and P-glycoprotein P-gp (known also as multidrug resistance MDR1), we combined it with remdesivir, which is a putative CYP3A and P-gp substrate. The drug combination was able to synergistically rescue the viability of infected cells to levels comparable to uninfected controls and to almost entirely abrogate viral replication in two cell lines and a primary colon organoid (Figure 1B-D).

Conclusion: Cobicistat and remdesivir synergistically inhibit SARS-CoV-2 replication and cytopathic effects. Cobicistat can form the backbone of combination treatments due to its dual activity as direct antiviral and pharmacoenhancer.
ACUTE KIDNEY INJURY IN PATIENTS WITH MODERATE COVID-19 TREATED WITH RDV VERSUS SoC

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Background: Remdesivir (RDV), an RNA-dependent RNA polymerase inhibitor of SARS-CoV-2, and its intravenous formulation, cyclophosphamide, are renally cleared. We sought to characterize whether RDV was associated with worsening renal function in hospitalized patients with moderate COVID-19.

Methods: We conducted an open-label, phase 3 trial (NCT04252664) involving hospitalized patients with confirmed SARS-CoV-2 infection, evidence of pulmonary infiltrates, oxygen saturation >94% on room air and eGFR ≥50 mL/min. Patients were randomly assigned 1:1:1 to receive up to 5d or 10d RDV. RDV was dosed intravenously at 200 mg on d1 and 100 mg daily thereafter.

Results: 1005 patients (822 [83%] RDV, 183 [17%] SoC) with creatinine values collected through d14 were evaluated. Baseline patient demographics, comorbidities, creatinine, and eGFR were mostly similar between RDV vs SoC arms. Worsening renal function was observed less frequently in patients receiving RDV vs SoC (7% vs 10%, p=0.03, Table). After adjustment for age, there was no significant association of RDV with risk of AKI relative to SoC (RR=0.66; 95% CI 0.40, 1.09). Most AKI events were observed in patients with baseline eGFR >50 mL/min, with few events occurring in patients with a baseline eGFR 50-59 mL/min.

Conclusion: AKI events were observed less frequently in patients with moderately severe COVID-19 patients treated with RDV compared to SoC.
TREATMENT AND OUTCOMES OF COVID-19 IN THE US: ARE THEY DIFFERENT ACCORDING TO RACE?

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Background: Clinical practice patterns for hospitalized COVID-19 patients have rapidly evolved, including specific treatment utilization. In turn, outcomes including time to improvement and mortality have also changed, but some reports have shown disproportionate mortality in Blacks. Data on the use of COVID-19 treatments over time and temporal association with hospital mortality and length of stay (LOS), along with assessments by race, are lacking.

Methods: This was a retrospective cohort study of adult patients with a discharge diagnosis of COVID-19 (ICD-10-CM: U07.1) admitted between May–Nov 2020 using the chargemaster inpatient data from the Premier Healthcare Database. Demographic characteristics of the cohort were summarized. Utilization of remdesivir (RDV), dexamethasone, anticoagulants, tocilizumab, sarilumab and baricitinib were examined. Median hospital and intensive care unit (ICU) LOS were assessed over time. In-hospital mortality was identified through discharge status. Unadjusted mortality rates over time are reported.

Results: Between May–Nov 2020, 190,529 patients were hospitalized for COVID-19 in 823 US hospitals. Patients had a mean age of 64 years; 64% were White, 19% Black, 53% male and 65% had Medicare/Medicaid as primary payor. Black patients were younger than White (mean 60 vs. 66 years). Significant comorbidities (≥20%) were similar between overall cohort and Black patients and included chronic pulmonary disease, hypertension and obesity. From May to Nov, overall RDV utilization increased from 5% to 47%, dexamethasone utilization increased from 7% to 77% and anticoagulant treatment utilization decreased from 32% to 24% (Figure). Few patients received tocilizumab (5%), sarilumab (0.02%) and baricitinib (0.003%). Among Black patients, RDV use decreased from 32% to 24% (Figure). Few patients received tocilizumab (5%), utilization increased from 7% to 77% and anticoagulant treatment utilization decreased from 32% to 24% (Figure). Few patients received tocilizumab, sarilumab and baricitinib were examined. Median hospital and intensive care unit (ICU) LOS were assessed over time. In-hospital mortality was identified through discharge status. Unadjusted mortality rates over time are reported.

Conclusion: In US hospitalized patients, use of both dexamethasone and RDV has increased approximately 10-fold from May to Nov. Over this same time, a 35% reduction in mortality, a 17% reduction in LOS and 20% reduction in ICU stay were observed. Besides age, no notable differences were apparent by race. Understanding the drivers of improvement in outcomes requires further analyses.

TENOFOVIR DIPHOSPHATE IN DRIED BLOOD SPOTS PREDICTS FUTURE VIREMIA IN SOUTH AFRICA

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Background: Tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) is a powerful biomarker of cumulative ART adherence. While some data demonstrate the value of this measure to predict future viremia, no data in African persons living with HIV (PLWH) are available. We examined the ability of TFV-DP in DBS to predict future viral breakthrough in South African PLWH.

Methods: We enrolled 250 PLWH (≥18 years of age) from 4 primary health clinics in Cape Town receiving tenofovir disoproxil fumarate (TDF)-based regimens (for 4 to 24 months) who had an undetectable (<50 copies/mL) HIV viral load (VL). Paired HIV VL and DBS for TFV-DP were collected monthly for 12 months. Viral breakthrough was defined as the first HIV VL >400 copies/mL. Receiver operating characteristics (ROC) analysis identified the TFV-DP threshold that best predicted viral breakthrough at the next monthly visit and generalized estimating equations to estimate the odds ratio and 95% confidence intervals (95% CI) for this association.

Results: Participants provided 2,944 paired DBS and HIV VL samples. Mean (SD) age was 35.52 (10.42) years; mean duration on ART at study entry was 10 (5) months; 78% were women. Median (IQR) study visits completed was 13 (12,13). Median overall TFV-DP concentration was 1,041 (727,1355) fmol/punch. Twenty-one participants developed viral breakthrough, with a median VL of 9,505 (1,430,45,481) copies/mL, and TFV-DP concentrations of 241 (49,417) fmol/punch at the first breakthrough (Figure). A threshold TFV-DP concentration in DBS of 400 fmol/punch maximized sensitivity and specificity to detect future viral breakthrough. Participants with TFV-DP ≤400 fmol/punch had 12 times the odds (95% CI: 18, 57; p<0.001) of developing future viral breakthrough one month later compared to participants with TFV-DP >400 fmol/punch.

Conclusion: TFV-DP in DBS strongly predicted future viral breakthrough the following month in South African PLWH. These results are consistent with data established in US PLWH, although TFV-DP threshold concentrations were lower in this population, possibly due to biologic differences in study populations, use
EFFECTIVENESS OF THE DOLUTEGRAVIR TRANSITION IN UGANDA: DISCO COHORT WEEK-24 RESULTS

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1 Massachusetts General Hospital, Boston, MA, USA, 2 Mbarara University of Science and Technology, Mbarara, Uganda, 3 Harvard Medical School, Boston, MA, USA, 4 Emory University, Atlanta, GA, USA, 5 University of KwaZulu-Natal, Durban, South Africa, 6 University College London, London, UK, 7 Cambridge University, Cambridge, UK

Background: The fixed-dose combination of tenofovir (TFD), lamivudine (3TC), and dolutegravir (DTG) is now preferred first-line antiretroviral therapy (ART) for most adults with HIV in Sub-Saharan Africa. Yet, concerns remain about durability of TLD with high circulating resistance to 3TC and TDF and metabolic abnormalities observed in clinical trials. Limited programmatic data are available to describe the success of the TLD transition in the region.

Methods: We established the DISCO cohort to quantify viral suppression and regimen tolerability during the TLD transition. We prospectively enrolled adults from public clinics in Uganda and South Africa who had been on non-nucleoside reverse transcriptase inhibitor-based ART for ≥6 months and were programatically switched to TLD. We obtained demographics, medical history data, and plasma specimens at enrollment and week 24. We conducted retrospective HIV-1 RNA viral load (VL) testing using the Cepheid GeneXpert platform. Though both sites were interrupted by COVID-19, here we report complete week 24 results for the Uganda cohort.

Results: We enrolled 500 participants (41% female) in Uganda. Median age was 47 years (IQR 40 – 53). Median ART duration was 8.8 years (IQR 5.7 – 12.2). The most common regimens prior to TLD switch were 3TC/TDF/efavirenz (44%) and 3TC/zidovudine/nevirapine (39%). Retrospective VL testing demonstrated that 95% (475/499) had VL <50 copies/mL, 4% (19/499) had VL 50–1,000 copies/mL, and 1% (5/499) had VL >1,000 copies/mL at enrollment. 90% (448/500) completed week 24 visits, with 50 additional visits delayed during COVID-19. 95% (475/499) had VL <50 copies/mL, 4% (19/499) had VL 50-1,000 copies/mL, and 1% (4/499) had VL >1,000 copies/mL at week 24. Of those with week 24 VL >50 copies/mL, 31% (5/16) had VL <1,000 copies/mL, 3% (12/448) had VL 50–1,000 copies/mL, and 1% (4/448) had VL >1,000 copies/mL. Of those with week 24 VL >50 copies/mL, 31% (5/16) had detectable VL at week 24 (χ2 p-value<0.001).

Conclusion: The great majority of participants transitioned to TLD with an undetectable VL. Overall, we documented 86% suppression at week 24 after TLD switch in the midst of the COVID-19 pandemic and 96% suppression in those completing a week 24 visit. These data support early tolerability and efficacy of TLD transition in the public sector. However, detectable VL at switch predicted detectable VL at 24 weeks. Vigilance and programmatic monitoring are needed to ensure long-term durability of TLD.

Dolutegravir in Real Life: Quality-of-Life Outcomes in a Cohort Study in Lesotho

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1 Swiss Tropical and Public Health Institute, Basel, Switzerland, 2 SolidarMed, Maseru, Lesotho, 3 Butha-Buthe Government Hospital, Maseru, Lesotho, 4 Ministry of Health, Maseru, Lesotho, 5 University of Maryland, College Park, College Park, MD, USA, 6 University of Basel, Basel, Switzerland

Background: Following the World Health Organization 2018 interim guidance, HIV programs in low-resource settings routinely transition individuals taking efavirenz (EFV) based first-line antiretroviral therapy (ART) to dolutegravir (DTG) containing ART. As both drugs are associated with neuropsychological side-effects, this prospective cohort study assesses mental health as well as common HIV/ART-related symptoms before and after transition from EFV to DTG in Lesotho.

Methods: The Dolutegravir in Real Life in Lesotho cohort enrols people living with HIV transitioning to or initiating DTG-based therapy (NCT04238376). Here, we report results of adult participants undergoing a programmatic transition from EFV to DTG at Butha-Buthe Government Hospital, Lesotho. At baseline (day of transition from EFV to DTG) and follow-up (16 weeks [10-24 weeks] after transition), participants were interviewed using the Patient Health Questionnaire-9 (PHQ-9) to screen for depression, and a modified HIV symptom index (mHSI) questionnaire containing 21 pre-specified symptoms. Enrolment began on Feb 10, 2020 and data was closed for this analysis on Nov 16, 2020. Differences in PHQ-9 outcomes and mHSI symptoms before and after the transition were assessed using the marginal homogeneity test and the McNemar test, respectively. In addition, we report results stratified by gender.

Results: At data closure, 664 participants had completed follow-up, 339/664 (60.1%) were female, median age was 47 years (IQR 38-56), and median time on ART was 5.5 years (IQR 3.3-8.8). Baseline and follow-up PHQ-9 data were available for 662/664 participants. In both genders, the proportion reporting at least mild depression symptoms (score ≥5) nearly halved after transition to DTG (59/661 (8.9%) vs 32/661 (4.8%); table). mHSI data were available for 649/664 participants. The sum of reported symptoms across all individuals decreased from 821 at baseline to 597 at follow-up. The frequency of reporting changed significantly in five mHSI symptoms, all of which decreased after transition to DTG. The greatest change was observed for feeling nervous/anxious (table).

Conclusion: In this ART-experienced adult population in Lesotho, the prevalence of reported depressive as well as psychosomatic symptoms decreased after routine transition from EFV to DTG-containing ART. The WHO recommendation to shift from EFV to DTG as first-line ART in low-resource settings may thus not only improve viral suppression but also quality of life among persons living with HIV.
401 WEEK 96 EFFICACY AND SAFETY OF CABOTEGRAVIR + RILPIVIRINE EVERY 2 MONTHS: ATLAS-2M


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Background: The dosing frequency of cabotegravir (CAB) and rilpivirine (RPV) long-acting (LA) administered every 1 or 2 months may address challenges associated with daily oral ART, such as adherence, pill burden, and stigma. The Week (W) 48 results from ATLAS-2M (NCT02929049) demonstrated noninferiority of CAB+RPV LA administered every 8 weeks (Q8W) compared with every 4 weeks (Q4W). Here, we report the W96 results.

Methods: ATLAS-2M was an ongoing Phase 3b, randomized, multicenter study assessing the efficacy and safety of CAB+RPV LA Q8W vs. Q4W. Virologically suppressed individuals receiving CAB+RPV LA Q4W (ATLAS [NCT02951052] study rollover) or oral SoC were randomized 1:1 to receive CAB+RPV LA Q8W or Q4W. The primary endpoint at W48 was the proportion of participants with plasma HIV-1 RNA ≤50 c/mL (FDA Snapshot, ITT-E; 12% noninferiority margin). Endpoints assessed at W96 include proportion of participants with plasma HIV-1 RNA ≤50 c/mL and HIV-1 RNA ≤50 c/mL, incidence of confirmed virologic failure (CVF; two consecutive plasma HIV-1 RNA ≥200 c/mL), safety, and tolerability.

Results: 1045 participants received CAB+RPV LA Q8W (n=522; Q4W, n=523). The median (range) age was 42y (19–83); 27% were female (sex at birth), and 73% were white. At W96, CAB+RPV LA Q8W confirmed noninferiority to Q4W dosing, with 2.1% (n=11) and 1.1% (n=6) of participants having HIV-1 RNA ≥50 c/mL in each arm, respectively (Table 1). High levels of virologic suppression were observed across both arms, with 90–91% of participants maintaining HIV-1 RNA <50 c/mL at W96. Through W96, 9.1% (Q8W) and 2.0% (Q4W) participants in the Q8W and Q4W arms had CVF, respectively; 1 occurred between W48 and W96 (participant in Q8W arm with baseline RPV resistance-associated mutation [RAM] Y181C and no InSTI RAMs). Safety profiles were comparable between age groups (Q8W W48: ≥50y, 5.0 [9.18]; <50y, 4.8 [9.13]; Q4W W48: ≥50y, 5.6 [9.50]; <50y, 4.0 [9.43]; SoC W48: ≥50y, 0.4 [7.36]; <50y, 0.7 [9.02]).

Conclusion: Efficacy of CAB+RPV LA Q8W continued to be noninferior to Q4W at W96, with both regimens maintaining high levels of virologic suppression. These longer-term efficacy, safety, and tolerability data further support the therapeutic potential of CAB+RPV LA.

Table 1. ATLAS-2M Key Results at Week 48 and Week 96 Analysis Timepoints

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Week 48</th>
<th>Week 96</th>
</tr>
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<tbody>
<tr>
<td><strong>Primary endpoint (Snapshot based on the ITT-E population)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA ≤50 c/mL, fDA Snapshot</td>
<td>9 (1.7)</td>
<td>6 (1.1)</td>
</tr>
<tr>
<td><strong>Adjusted difference (95% CI)</strong></td>
<td>0.8 (–0.6 to 2.2)</td>
<td>1.0 (–0.6 to 2.6)</td>
</tr>
</tbody>
</table>

*Data in window not <50 c/mL* Discontinued for lack of efficacy Discontinued for other reason while not <50 c/mL

<table>
<thead>
<tr>
<th><strong>Secondary endpoints</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>HIV-1 RNA ≤50 c/mL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Confirmed virologic failure</strong></td>
<td>3 (6.0)</td>
<td>1.0 (2.0)</td>
</tr>
<tr>
<td><strong>Confirmed virologic failure</strong></td>
<td>1.0 (2.0)</td>
<td>0.2 (0.4)</td>
</tr>
<tr>
<td><strong>Confirmed virologic failure</strong></td>
<td>0.2 (0.4)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>Confirmed virologic failure</strong></td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

*No discontinuations were attributed to COVID-19. Missing virologic data for 4 on-study participants were deemed to be COVID-19 related. COVID-19 introduced negligible impact on efficacy and no impact on the conclusions drawn at Week 96.*

1Adjusted for prior exposure to CAB+RPV.
2Day 1 to Week 100.

402 LONG-ACTING CABOTEGRAVIR + RILPIVIRINE IN OLDER ADULTS: POOLED PHASE 3 WEEK-48 RESULTS

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Background: Cabotegravir (CAB) and rilpivirine (RPV) long-acting (LA) dosed intramuscularly every 4 weeks (Q4W) was noninferior to daily oral standard of care (SoC) in the ATLAS (NCT02929052) and FLAIR (NCT02938520) Phase 3 studies. CAB+RPV LA dosed every 8 weeks (Q8W) was noninferior to Q4W dosing in the Phase 3b ATLAS-2M study (NCT02929049). Owing to the benefits of ART, there is an increasing proportion of people living with HIV (PLWH) aged ≥50y. Efficacy, safety, adherence, and treatment satisfaction outcomes stratified by age (≥50y and <50y) across the ATLAS, FLAIR, and ATLAS-2M studies at Week (W) 48 are reported.

Methods: Pooled data from the three studies were stratified by age (≥50y and <50y). For participants in ATLAS-2M who transitioned from ATLAS LA therapy, only data from ATLAS were included. W48 primary and secondary efficacy endpoints were the proportion of participants with plasma HIV-1 RNA ≤50 c/mL (virologic nonresponse) and HIV-1 RNA <50 c/mL (virologic suppression), respectively (FDA Snapshot, intention-to-treat exposed). Efficacy, safety, adherence, and treatment satisfaction outcomes stratified by age (≥50y and <50y) across the ATLAS, FLAIR, and ATLAS-2M studies at Week (W) 48 are reported.

Results: In total, 399 aged ≥50y and 1437 participants aged <50y were randomized to either CAB+RPV LA Q8W (≥50y, n=185; <50y, n=238), CAB+RPV LA (≥50y, n=185; <50y, n=733), or SoC (≥50y, n=125; <50y, n=466). Table 1 shows baseline characteristics and key outcomes. Virologic outcomes were similar across arms and age groups; rates of virologic suppression were high (~92–97%) and rates of nonresponse were low (~2%). CVF rates were similarly low across arms and age groups. Safety profiles between participants ≥50y and <50y were similar for both LA regimens; few adverse events led to withdrawal. Injection site reactions were similar in frequency and severity across LA arms and age groups, with a median duration of 3 days. Mean change (SD) from baseline in total treatment satisfaction was higher in the LA arms vs. SoC but was comparable between age groups (Q8W W48: ≥50y, 5.0 [9.18]; <50y, 4.8 [9.96]; Q4W W44: ≥50y, 5.6 [9.50]; <50y, 4.0 [9.43]; SoC W44: ≥50y, 0.4 [7.36]; <50y, 0.7 [9.02]).
Conclusion: CAB+RPV LA demonstrated similar efficacy, safety, and tolerability between participants aged ≥50y and <50y. Treatment satisfaction improved from baseline with CAB+RPV LA and was comparable by age. These data support the therapeutic potential of CAB+RPV LA in older PLWH.

Table 1. Pooled Outcomes From ATLAS, FLAIR, and ATLAS-2M Stratified by Age (≥50y and <50y)

<table>
<thead>
<tr>
<th>Group</th>
<th>CAB + RPV LA vs. Q2M</th>
<th>CAB + RPV LA vs. Q2M</th>
<th>Oral BIC</th>
<th>Oral BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>44 (0.16)</td>
<td>12 (0.05)</td>
<td>22 (0.09)</td>
<td>11 (0.04)</td>
</tr>
<tr>
<td>n (%)</td>
<td>248 (48)</td>
<td>60 (12)</td>
<td>188 (37)</td>
<td>40 (8)</td>
</tr>
</tbody>
</table>

CAB, rilpivirine; Q2M, every 2 months; BIC, bictegravir; BIC LA, self-administered; BIC IM, injection; CAB LA, self-administered; CAB IM, injection; BIC vs CAB, bictegravir vs cabotegravir.

403 POPPK MODELING OF Q2M IM RPV LA FOR MANAGING DOSING INTERRUPTIONS IN HIV-1 PATIENTS

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Background: Long-acting rilpivirine (RPV LA) is intended for coadministration with cabotegravir long-acting (CAB LA) as a complete 2-drug injectable regimen for HIV-1 treatment. Eight-weekly RPV LA plus CAB LA was noninferior to 4-weekly RPV LA plus CAB LA in maintaining HIV-1 suppression (ATLAS-2M; Overton TE et al. CROI 2020 Abstract 34).

Methods: Every 2 months (Q2M) RPV LA consists of 1-month oral RPV 25mg once daily for tolerability assessment, two initiation RPV LA 900mg (3mL) intramuscular (IM) doses separated by 1 month and subsequent 900mg doses Q2M, to be administered with the Q2M CAB regimen. Population pharmacokinetic (PopPK) modeling and simulation was used to inform strategies for managing dosing interruptions, aimed at minimizing impact on the overall RPV LA PK profile. Simulations included effects on RPV concentrations of Q2M vs 8-weekly dosing, IM dosing delays, and bridging with oral RPV to cover a planned missed IM injection. Simulated RPV concentrations on the overall RPV LA PK profile. Simulations included effects on RPV

Results: Simulated RPV plasma concentration-time profiles of Bridging With Oral RPV 25 mg qd during Planned RPV LA Dosing Interruptions for 1 Month (And Resuming With RPV LA 900 mg Every 2 Months; Upper Panel) and 2 Months (Re-initiating with RPV LA 900 mg. 1 Month Later Another RPV LA 900 mg. Then RPV LA 900 mg Every 2 Months)

404 BICTEGRAVIR AND CABOTEGRAVIR: IN VITRO PHENOTYPIC SUSCEPTIBILITY OF HIV-1 NONGROUP M

Charlène Martin1, Ségoïlène Gracias1, Charlotte Charpentier2, Diane Descamps2, Quentin Le Hingrat1, Jean-Christophe Plantier1, Elodie Alessandri-Gradt1, Janssen, Beerse, Belgium, 2Janssen Pharmaceutical, Research and Development, Titusville, FL, USA

Background: HIV-1 are classified into 4 groups: M (pandemic), O (endemic in Cameroon), N and P (more rare). The WHO has recommended the use of integrase strand transfer inhibitors (InSTIs) as first line treatment. Previous phenotypic studies showed the susceptibility of HIV-1/non-M to raltegravir and dolutegravir and a more variable susceptibility to elvitegravir, in association with a probable specific genotypic pattern. This study was conducted to evaluate the phenotypic susceptibility of a large panel of HIV-1/non-M to the newest InSTIs, bictegravir (BIC) and cabotegravir (CAB), which are promising molecules regarding the limited therapeutic arsenal available for HIV-1/non-M infected patients.

Methods: 44 clinical isolates of HIV-1/non-M (41 O, 2 N and 1 P) were tested and 5 isolates of HIV-1/M from cell culture were used as standard references. The phenotypic assay was performed by infecting fresh PBMCs with a probable specific genotypic pattern. This study was conducted to evaluate the phenotypic susceptibility of a large panel of HIV-1/non-M to the newest InSTIs, bictegravir (BIC) and cabotegravir (CAB), which are promising molecules regarding the limited therapeutic arsenal available for HIV-1/non-M infected patients.

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1Janssen, Beerse, Belgium, 2Janssen Pharmaceutical, Research and Development, Titusville, FL, USA

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Methods: 44 clinical isolates of HIV-1/non-M (41 O, 2 N and 1 P) were tested and 5 isolates of HIV-1/M from cell culture were used as standard references. The phenotypic assay was performed by infecting fresh PBMCs with non-M supernatants for 2 hours and adding 5 increasing concentrations of the drug (0.1 nM – 1000 nM) in quadruplicates for 3 days. The viral material was quantified (qRT-PCR) for the different conditions and inhibitory concentrations 50% (IC50) were compared to the 5th percentile of observed RPV concentrations 4 weeks after the initial RPV LA 900mg dose in ATLAS/FLAIR and to concentrations on the overall RPV development program, as done for monthly RPV LA (Rossenu S et al. AIDS 2020 PEB0264).

Results: Q2M vs 8-weekly dosing, with a 7-day window, has minimal impact on overall RPV PK profile. IM dosing delays of >7 days may have a larger impact, particularly in the first few months of therapy. If a patient plans to miss a scheduled injection by >7 days, oral RPV can provide coverage of up to 1 missed injection (Figure 1). Recommendations for resuming RPV LA after missed injections are: if time since last injection: ≤2 months (Injection 2) or ≤3 months (Injection 3 or later): re-initiate with 900mg dose (3mL) followed by a second 900mg dose (3mL) one month later, then continue Q2M dosing. Conclusion: Adherence to the Q2M RPV LA injection schedule is strongly recommended. Oral therapy to cover planned dosing interruptions of RPV LA injections can provide exposures within ranges observed in clinical studies. Recommendations for managing dosing interruptions are aligned for RPV LA and CAB LA, to facilitate dosing for the complete regimen.
especially with injectable CAB in patients. Clinical studies of in vivo efficacy are still required to confirm these promising phenotypic results.

**405 IMPACT OF ANTIRETROVIRAL REGIMENS ON MORTALITY IN PATIENTS WITH ADVANCED HIV DISEASE**

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**Background:** Scarce data exist regarding the efficacy of antiretroviral (ARV) treatment in patients with advanced HIV disease. The aim of the study was to assess the impact of ARV regimens on the clinical outcomes among naive patients with advanced HIV presentation in real life settings.

**Methods:** A multicentre, population-based, prospective cohort study was performed. Treatment-naive subjects with advanced HIV diseases (CD4+ T cell count < 200 cells/ml or presence of an AIDS-defining illness) who started treatment between 2010 and 2018, from 18 hospitals in Spain, were included. The primary outcome was the rate of mortality at three years. Secondary outcomes included discontinuation or change of ARV regimen, virological effectiveness (viral load of ≤ 200 copies/ml) and immune reconstitution (achieve CD4+ T cell count > 350 cells/ml). Kaplan-Meier curves and long-rank test were used to analyse different outcomes. A Cox proportional hazard model was performed to identify predictors of death.

**Results:** A total of 1170 naive patients with advanced HIV disease started ARV treatment: 44.9% with PI-based regimen, 29.6% with NNRTI and 25.6% with INSTI. The most frequently third-drug was darunavir (73%), efavirenz (70.9%) and dolutegravir (47%), respectively. The median follow-up was 5 years (5695 person-years), median CD4+ T cell count at baseline was 101 cells/ml and 30.3% had an AIDS-defining illness. Crude mortality rate at three years of follow-up per 100 person-years [IR]: 12.54; 95% confidence interval [CI]: 9.94, 15.83) compared to other regimens (range IR: 14.5 to 35.27). While 70% reached a CD4 count ≥ 200 cells/μL overall, CD4:CD8 ratio normalization (≥ 1) was achieved by <7% and did not differ across regimens (logrank p = 0.52). Incident immune reconstitution inflammatory syndrome (IRIS) was rare (3 B/F/TAF, 1 bDRV, 2 DTG, 0 EVG/c). Follow-up viral loads were available for 762 PLWH (355 B/F/TAF, 75 bDRV, 218 DTG, 114 EVG/c). Baseline characteristics were well balanced with inverse probability of treatment weights. Compared to B/F/TAF, bDRV initiators were statistically significantly less likely to discontinue their regimen (incidence rate per 100 person-years [IR]: 12.54; 95% confidence interval [CI]: 9.94, 15.83) compared to other regimens (range IR: 14.5 to 35.27). Among PLWH with advanced HIV infection initiating ART, those on B/F/TAF appeared less likely to discontinue their regimen compared to other 3DRs (unadjusted) and were numerically more likely to achieve virologic suppression (adjusted hazard ratio: 0.75; 95% CI: 0.55, 1.02). No statistically significant difference in the likelihood of virologic suppression was detected between B/F/TAF and DTG or EVG/c-based 3DR (Figure).}

**Conclusion:** Among PLWH with advanced HIV infection initiating ART, those on B/F/TAF appeared less likely to discontinue their regimen compared to other 3DRs (unadjusted) and were numerically more likely to achieve virologic suppression compared to bDRV but did not differ from those on DTG or EVG/c-based 3DR (adjusted).

**406 EFFECTIVENESS OF RECOMMENDED 3-DRUG REGIMENS FOR TREATING ADVANCED HIV INFECTION**

Karam Mouneer1, Laurent Brunet1, Jennifer S. Fusco1, Ian McNicholl1, Helena Diaz-Cuervo1, Michael Senson2, Lewis McCurdy2, Gregory P. Fusco2

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**Background:** There is limited evidence on regimen options for people living with HIV (PLWH) initiating antiretroviral therapy (ART) with advanced infection. The effectiveness of one of the newest 3-drug regimen (3DR), bictegravir/tenofovir alafenamide (B/F/TAF), was therefore compared to other 3DRs that included boosted darunavir (bDRV), dolutegravir (DTG) or elvitegravir/cobicistat (EVG/c) among ART-naive PLWH with CD4 count <200 cells/μL.

**Methods:** ART-naive adults with advanced HIV-1 infection (CD4 count <200 cells/μL) initiating B/F/TAF or a bDRV, DTG or EVG/c-based 3DR between 01/2018 and 03/2019 in the OPERA Cohort were included. Regimen discontinuation and virologic suppression to ≤50 copies/ml were assessed with Kaplan-Meier methods and unadjusted Poisson regression. The association between regimen and virologic suppression was assessed with a Cox proportional hazards model with inverse probability of treatment weighting (Figure).

**Results:** Overall, 961 PLWH initiated ART with advanced HIV infection: 416 B/F/TAF (age ≤ 25: 10%, CD4 ≤ 0 cells/ml: 36%), 106 bDRV (age ≤ 25: 19%, CD4 ≤ 50%: 33%), 271 DTG (age ≤ 25: 13%, CD4 ≤ 50%: 30%), 168 EVG/c (age ≤ 25: 14%, CD4 ≤ 50%: 38%). In unadjusted analyses, B/F/TAF initiators were statistically significantly less likely to discontinue their regimen (incidence rate per 100 person-years [IR]): 12.54; 95% confidence interval [CI]: 9.94, 15.83) compared to other regimens (range IR: 14.5 to 35.27). While 70% reached a CD4 count ≥ 200 cells/μL overall, CD4:CD8 ratio normalization (≥ 1) was achieved by <7% and did not differ across regimens (logrank p = 0.52). Incident immune reconstitution inflammatory syndrome (IRIS) was rare (3 B/F/TAF, 1 bDRV, 2 DTG, 0 EVG/c). Follow-up viral loads were available for 762 PLWH (355 B/F/TAF, 75 bDRV, 218 DTG, 114 EVG/c). Baseline characteristics were well balanced with inverse probability of treatment weights. Compared to B/F/TAF, bDRV initiators were numerically less likely to achieve virologic suppression (adjusted hazard ratio: 0.75; 95% CI: 0.55, 1.02). No statistically significant difference in the likelihood of virologic suppression was detected between B/F/TAF and DTG or EVG/c 3DRs (Figure).

**Conclusion:** Among PLWH with advanced HIV infection initiating ART, those on B/F/TAF appeared less likely to discontinue their regimen compared to other 3DRs (unadjusted) and were numerically more likely to achieve virologic suppression compared to bDRV but did not differ from those on DTG or EVG/c-based 3DR (adjusted).
**407 BLIP INCIDENCE IN DOLUTEGRAVIR- OR EFAVRINZ-E BASED ART DURING ACUTE HIV INFECTION**

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**Background:** Transient viral blips are observed in up to 50% of persons living with HIV on ART. As first-line regimens shift from efavirenz (EFV) to dolutegravir (DTG), evaluation of blip incidence is needed. We compared blip incidence in participants diagnosed and started on either regimen during acute HIV infection (AHI) in Bangkok, Thailand.

**Methods:** From 2013-2018, participants with AHI in SEARCH010/RV254 cohort initiated EFV- or DTG-based regimens. Fiebig stage was assessed at enrolment using nucleic acid testing and sequential immunoassays. HIV RNA was measured at enrolment, weeks 2, 4, 8, 12 and every 12 weeks thereafter. Blip was defined as a transiently detectable RNA (≥20 copies/mL) bokomed by undetectable measurements with self-reported adherence of >95%. Blips were categorised as “low” (<20-50 copies/mL), “medium” (51-200 copies/mL) or “high” (>200 copies/mL). Blips were counted after achieving viral suppression with 2 consecutive undetectable RNA measurements.

**Results:** A total of 324 participants were analysed, predominantly MSM (98.5%) with a median age of 26 (IQR 22-31) years. Of these, 280 started EFV and 44 started DTG-based ART. Fifty-five blips were observed with an incidence of 11.5% (95% CI 8.7-15.3) per 100 person-years. Blip incidence was not statistically different between DTG and EFV group (15.5 vs 10.8, p=0.265). The frequency of blip in DTG and EFV were 11 vs 44 (p=0.041). The categories respectively were 6 (5%) vs 37 (84%) in low, 4 (36%) vs 7 (16%) in medium and 1 (9%) vs 0 in high. Blip range was 21-398 copies/mL in DTG and 21-160 copies/mL (EFV), while blip median was 34 (IQR 24-103) and 30 (IQR24-43) respectively (p=0.215). The median time from ART initiation to viral suppressions was 8 weeks (IQR 6-12) on DTG and 23 weeks (IQR 12-24) on EFV (p=0.001). The median time from ART initiation to first blip was 72 weeks in both groups. In the multivariate model, factors associated with higher incidence rate of blips are baseline HIV RNA>600 copies/mL and diagnosis at Fiebig stages III-V. CD4 count and ART regimen (EFV or DTG) were not associated with blip incidence rate ratio.

**Conclusion:** There is no significant trend of increasing frequency and magnitude of blips in DTG compared to EFV regimen. Time to viral suppression was faster with DTG and may have led to a longer time-at-risk for blips. Magnitude of blips in both regimens was low, suggesting a low risk of subsequent viral failure. Participants with baseline HIV RNA>100,000 copies/mL and Fiebig stages III-V at enrolment were predictive of blips.

**408 HMMCgag ASSAY DETECTS HIGH VIREMIA RATES ON ART STARTED DURING ACUTE HIV INFECTION**

Donn J. Colby, Suteeraporn Pinyakorn, Carlo Saldanlan, Adam Yates, Eugene Koon1, Denise C. Husi, Nittaya Phanuphak, Jintanat Ananworanich, Jeffrey Lifson, Brandie Fullmer, Jorden L. Welker, Robert Gorelick, Sandhya Vasan, Frank Maldarellia, for the RV254/SEARCH010 Research Group

US Military HIV Research Program, Bethesda, MD, USA, 1Institute of HIV Research and Innovation, Bangkok, Thailand, 2Armed Forces Research Institute of Medical Sciences in Bangkok, Bangkok, Thailand, 3University of Amsterdam, Amsterdam, Netherlands, 4Frederick National Laboratory for Cancer Research, Frederick, MD, USA, 5National Cancer Institute, Bethesda, MD, USA

**Background:** The goal of antiretroviral therapy (ART) for HIV infection is to suppress the plasma viral load (VL) to below the limit of detection (LOD) on commercial assays, thereby preventing adverse effects of HIV viremia. However, ultrasensitive assays, with LOD <0.3 copies/mL, can detect residual viremia in most chronically infected individuals despite many years of suppressive ART, reflecting HIV production from long-lived reservoirs. Levels of residual viremia in those initiating ART during acute HIV infection (AHI) are not known.

**Methods:** The RV254/SEARCH010 cohort has recruited participants with AHI in Bangkok, Thailand since 2009. Participants who started ART immediately at study entry, with VL<50 copies/mL at 24 and 48 weeks were included in the analysis. The HMMCgag single copy HIV-1 qRT-PCR assay was used for the plasma viral load analyses. This assay detects a relatively conserved target directly upstream of gag (Somsouk, PLoS One, 2014) and detects clades A through G.

**Results:** Participants (n=419) had median age 26 (interquartile range (IQR) 23-31) and were 98% male. At HIV diagnosis median (IQR) CD4 was 364 (266–490) cells/mm³, CD8 was 510 (335–857) cells/mm³, and the CD4/CD8 ratio was 0.71 (0.44-1.04). HIV subtypes were 76.9% CRF01_AE and 11.2% recombinant CRF01_AE/B. Initial ART regimens were efavirenz-based in 73.0% and contained an integrase inhibitor in 26.5%. Baseline median (IQR) VL was 5.94 (5.23-6.78) log₁₀ copies/mL in the commercial assay and 5.83 (5.06-6.54) log₁₀copies/mL in the HMMCgag assay, with high correlation (r=0.902, p<0.001). Although all participants had VL<6.0 log₁₀copies/mL, and time to VL suppression on ART >36 weeks (Table). Age, HIV subtype, CD4, CD8, CD4/CD8 ratio, and ART regimen were not associated with viremia.

**Conclusion:** Despite all participants having undetectable VL on the commercial assay, the majority had residual viremia on the HMMCgag assay, indicating persistence of a virus production through at least 48 weeks. Further studies are needed to determine the source and durability of this reservoir in participants who start ART during AHI.

**Table: Detectable HMMCgag at either week 24 or 48, by significant characteristics. All VL<LOD by commercial assay.**

<table>
<thead>
<tr>
<th>Fiebig stage</th>
<th>Detectable viremia at either weeks 24 or 48 (%)</th>
<th>aOR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiebig I</td>
<td>44</td>
<td>12 (29.4)</td>
<td>ref.</td>
<td>2.21</td>
</tr>
<tr>
<td>Fiebig II</td>
<td>77</td>
<td>64 (83.1)</td>
<td>3.69</td>
<td>2.23-6.09</td>
</tr>
<tr>
<td>Fiebig III</td>
<td>118</td>
<td>103 (87.3)</td>
<td>6.36</td>
<td>3.38-11.94</td>
</tr>
<tr>
<td>Fiebig IV-V</td>
<td>53</td>
<td>52 (98.1)</td>
<td>ref.</td>
<td>5.51</td>
</tr>
</tbody>
</table>

**409 CURRENT ANTIRETROVIRAL TREATMENT AMONG PEOPLE WITH HIV IN CARE IN THE US (2018-2019)**


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**Background:** Newer antiretroviral drugs and dosing formulations have expanded treatment options for people with HIV (PWH) in recent years. In a previous study, we found that the prevalence of PWH with limited antiretroviral treatment (ART) options declined dramatically to <2% after the introduction of the integrase strand transfer inhibitor (InSTI) class. Newer InSTIs, including dolutegravir (DTG) and bictegravir (BIC), have improved viral suppression and become the recommended “core” components in initial ART regimens. However, information regarding the uptake of newer drugs and current ART use patterns is lacking.

**Methods:** We studied all PWH aged 18 or older in routine clinical care 01/2018-12/2019 across the US in the GARF Network of Integrated Clinical Systems (CNICS) cohort. We examined current ART use, defined as the most recent multi-drug ART regimen, by core class (InSTI, non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI)), and by individual drugs in the overall cohort and among ART-naïve PWH initiating ART 2018-2019.

**Results:** Among 14,727 PWH in care, 96% were on ART, median age was 51 years, 20% were female, 61% non-white, and 55% men who had sex with men as a risk factor for HIV. Among PWH on ART, the majority were receiving InSTI-based regimens (72% of which 39% were anchored by BIC and 37% by DTG. A small proportion of PWH were on PI- (7%) and NNRTI- (9%) based regimens which on average were started 4 years prior to the study period (2014), with NNRTI use limited to single-tablet regimens (STRs, 99%). Sixty-nine percent of all regimens included tenofovir alafenamide (TAF) while only 10% used tenofovir disoproxil fumarate (TDF). Among 458 ART-naive PWH initiating treatment in 2018-2019, 93% were on InSTI-based regimens of which 73% were anchored by BIC and 23% by DTG.

**Conclusion:** The results of this study suggest high current uptake of ART nationally among PWH in care, predominance of newer InSTI-based regimens, and the emergence of BIC/TAF/FTC as the preferred regimen for both ART-experienced and naive individuals initiating ART. Few PWH remain on core agents with a lower barrier to resistance formulated as STRs. In addition, TAF...
has replaced TDF as the preferred form of tenofovir across all regimens. Further study of ART utilization patterns among specific subgroups of PWH is needed to guide development of targeted approaches to improve uptake and clinical outcomes for all PWH as the ART landscape continues to evolve rapidly.

<table>
<thead>
<tr>
<th>ART Regimen</th>
<th>Overall Median Start Year</th>
<th>First ART Initiated 01/2018-02/2019 (n = 2,413)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSTI-based *</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 409) (40%*)</td>
</tr>
<tr>
<td>M/TVDR/FTC (Triomune)</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 508) (30%*)</td>
</tr>
<tr>
<td>Delavirdine</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 140) (9%*)</td>
</tr>
<tr>
<td>ETV/STV/FTC (Savvy)</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 148) (10%*)</td>
</tr>
<tr>
<td>ETV/STV/FTC (Tival)</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 140) (9%*)</td>
</tr>
<tr>
<td>Duvatevir</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 140) (9%*)</td>
</tr>
<tr>
<td>Mozaven</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 148) (10%*)</td>
</tr>
<tr>
<td>NRTI-based *</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 140) (9%*)</td>
</tr>
<tr>
<td>Darunavir</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 148) (10%*)</td>
</tr>
<tr>
<td>Multiregime *</td>
<td>2016 (n = 1,449)</td>
<td>2018 (2018–2019) (n = 148) (10%*)</td>
</tr>
</tbody>
</table>

*Sub-group percentages are based on the number of patients. Sub-group percentages may not add up to 100.

**M/TVDR/FTC includes all tenofovir containing medications (M/TVDR/FTC, maraviroc/TVDR/FTC, and cobicistat/TVDR/FTC).

Conclusion: One-fifth of ART initiators experienced viremia within 2 years of ART, leading to an ART change was rare and more likely for patients initiating NNRTI-based regimens. Efforts to prevent viremia post-ART initiation should promote continuous ART and care engagement.

**Figure 1.** Stacked cumulative incidence curves of virologic failure (VF) by category among 6810 ART initiators in the US, 2008-2018.
412 DOLORTEGRAVIR VS DARUNAVIR/R-BASED ART IN VERY ADVANCED PATIENTS: 48-WEEK RESULTS

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1 Hospital Clinic Barcelona, Barcelona, Spain, 2 Hospital Universitario de Bellvitge, Barcelona, Spain, 3 Hospital de Mataró, Barcelona, Spain, 4 Hospital Germans Trias i Pujol, Barcelona, Spain, 5 Hospital Universitario de Bellvitge, Barcelona, Spain, 6 Hospital Gerners Trias i Pujol, Barcelona, Spain, 7 Hospital Universitario Virgen de la Victoria, Málaga, Spain, 8 Red de Investigación en SIDA, Barcelona, Spain

Background: Information on the impact of dolutegravir (DTG)-based antiretroviral therapy (ART) in very advanced patients is limited in terms of clinical, immunological and virological outcomes, bacterial translocation, inflammation and immune activation. Also, whether the impact on bacterial translocation varies with the ART regimen type is unknown, as boosted protease inhibitors (bPI) and integrase inhibitors (InSTI) have different effects on bacterial translocation, inflammation, immune activation, adverse events, IRIS, HIV disease progression and death. A mITT analysis was done (3 patients in the DRV/r arm were excluded. None started the study medication). Statistical analysis was performed using SAS v9.4 (SAS Inst. Inc., Cary, NC, USA).

Results: Baseline epidemiological, clinical, immunological and virological features and main results are depicted in the table. Median (IQR) increase in CD4 cell count at week 48 was 172 (118, 255) and 157 (66, 277) cells/mm³ in the DTG and DRV/r arms, respectively (p=0.8). There were four virological failures (1 in the DRV/r arm and 3 in the DTG arm). Inflammation (TNF-α, IL-6, hsCRP), immune activation (CD6+CD38+ T cells, CD8+CD38+DR+) and apoptotic markers (annexin-V) were similar at baseline and declined significantly and similarly in both ART arms (p>0.05 for all comparisons). A greater reduction in shCD14 marker in patients treated with DTG was found (802 [-1302, 2298] vs. 592 [-924, 000] ng/ml; p=0.011).

Conclusions: DTG-based ART was as effective as and had fewer discontinuations than DRV/r-based ART in very advanced ART-naïve HIV-1-infected patients and superior to the bPI regimen in reducing the bacterial translocation

**CMV infection**

<table>
<thead>
<tr>
<th>mITT analysis</th>
<th>Darunavir/r (n=52)</th>
<th>D/C/F/TAF (n=52)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr, median (IQR)</td>
<td>42 (38, 44)</td>
<td>42 (38, 44)</td>
<td>NA*</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>44 (87)</td>
<td>46 (89)</td>
<td>0.65</td>
</tr>
<tr>
<td>BMI, kg/m² at baseline, median (IQR)</td>
<td>26 (25, 31)</td>
<td>26 (25, 31)</td>
<td>0.33</td>
</tr>
<tr>
<td>Baseline AIDS-defining events (ADES), n (%)</td>
<td>22 (42)</td>
<td>24 (46)</td>
<td>0.24</td>
</tr>
<tr>
<td>Baseline RNA viral load (VL), median (IQR) log¹⁰ copies/mL</td>
<td>3.47 (3.24, 3.68)</td>
<td>3.64 (3.46, 3.12)</td>
<td>0.06</td>
</tr>
<tr>
<td>ART median duration (IQR)</td>
<td>41 (38, 47)</td>
<td>30 (11, 54)</td>
<td>0.002</td>
</tr>
<tr>
<td>48-wk. CD4 increase (median delta, IQR)</td>
<td>172 (118, 225)</td>
<td>157 (66, 277)</td>
<td>0.40</td>
</tr>
<tr>
<td>48-wk. ART-naïve VL &lt;50 copies/ml, n (%)</td>
<td>40 (77)</td>
<td>39 (70)</td>
<td>0.13</td>
</tr>
<tr>
<td>ART, n (%)</td>
<td>5 (10)</td>
<td>6 (12)</td>
<td>0.81</td>
</tr>
<tr>
<td>New ADEs/ith, n (%)</td>
<td>4 (8)</td>
<td>6 (12)</td>
<td>0.58</td>
</tr>
<tr>
<td>Treatment discontinuation (any reason), n (%)</td>
<td>4 (8)</td>
<td>8 (16)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

*NA: not applicable for baseline measurements

**413** D/C/F/TAF VS DTG/ABC/3TC FOR INITIAL TREATMENT IN HIV+ ADULTS: A RANDOMIZED STUDY

Daniel Podzamczer, Rafael Micán, Juan M. Tiraboschi, Joaquín Portilla, Pere Domingo, Josep Maria Llibre, Esther Ribera, Maria Jesus Vivancos, Luis Morano, Mar Masía, Cristina Gómez-Ayerbe, Antonio Navarro, Ana Caicedo, Santiago Moreno, for the SYMTRI Study Group (PreEC/RIS-57)

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Background: Integrase inhibitors (InSTI) are considered the preferred core for initial ART in HIV-infected pts. Darunavir/cobicistat/emtricitabine/tenofovir alafenamide (D/C/F/TAF) is an alternative regimen with high efficacy rates that has never been compared with InSTI-based regimens in randomized trials. We performed a head to head comparison between D/C/F/TAF and dolutegravir/abacavir/lamivudine (DTG/ABC/3TC) in ARV-naïve pts, both administered as single tablet regiments (STR).

Methods: Adults (> 18y) HIV-infected naïve pts (HLA B5701 and HBV negative), with viral load (VL) ≥ 500 c/mL from 27 Spanish hospitals, were randomized after stratifying by viral load (< or ≥ 100,000 c/mL) and CD4 cells (< or ≥ 200 cells/μL) between September 2018 and 2019. Clinical and analitical assessments were performed at weeks 0, 4, 12, 24 and 48. Primary endpoint was VL < 50 c/mL at week 48 (ITT exposed (ITTe), snapshot analysis, with a non-inferiority margin of 10%). Calculated sample size (10% lost to FU) was 316 pts. (EudraCT 2018-001645-14)

Results: Ten pts did not come back after enrollment visit (7 in D/C/F/TAF vs 3 in DTG/ABC/3TC arms). Groups were well balanced in the baseline characteristics and 306 pts were included in the ITTe analysis (151 and 155). 94% were male, median age 35 years, 79% were MSM, median VL 64,848 c/mL (40% > 100,000 c/mL), CD4 406/μL (13% < 200/μL, 27% 200-350), HCY 3% (N=73, BMI 24 kg/m², At 48 weeks, 7% of pts (DC/FR/TAF) vs 82% of DTG/ABC/3TC had VL < 50 c/mL (difference -2.4%, 95%CI -13 to 11.6). Virologic failure was 8% vs 4%; drug discontinuation due to adverse events was 4% (n=6, 5 skin rashes, 1 pulmonary TB) vs 6% (n=9, 3 neuropsychiatric symptoms, 2 muscle complaints, 2 gastrointestinal, 1 skin rash, 1 neoplasm); lost to follow-up 8% in each arm.

In the per protocol analysis (pts reaching 48 weeks with the allocated drug for at least 80% of scheduled time for at least 48 weeks), 93% were included in the per protocol analysis (PPA) with a non-inferiority margin of 10%. Calculated sample size (10% lost to FU) was 316 pts (EudraCT 2018-001645-14)

Conclusions: DTG-based ART was as effective as and had fewer discontinuations than DRV/r-based ART in very advanced ART-naïve HIV-1-infected patients and superior to the bPI regimen in reducing the bacterial translocation.
414 DURABLE EFFICACY OF DTG+3TC IN GEMINI-1&2: YEAR 3 SUBGROUP ANALYSES

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Background: In the GEMINI-1 & GEMINI-2 studies (ClinicalTrials.gov: NCT02831673 & NCT02831746), dolutegravir + lamivudine (DTG + 3TC) was non-inferior to the 3-drug regimen of DTG + tenofovir/emtricitabine (TDF/FTC) in achieving plasma HIV-1 RNA <50 c/mL in treatment-naive adults at Weeks 48, 96 and 144.

Methods: GEMINI-1&2 are identical, global, double-blind, multicenter Phase III studies. Participants with screening HIV-1 RNA ≤500,000 c/mL and no major viral resistance mutations to NRTIs, NNRTIs or PIs were randomized to once-daily DTG + 3TC or DTG + TDF/FTC, stratified by plasma HIV-1 RNA and CD4+ cell count. The primary endpoint was the proportion of participants with plasma HIV-1 RNA <50 c/mL at Week 48 (Snapshot algorithm). We present a secondary endpoint analysis of efficacy at Week 144 by baseline disease and demographic characteristics. For the overall population, estimates and confidence intervals were based on a stratified analysis using Cochran-Mantel-Haenszel weights.

Results: 714 and 719 adults were randomised and treated in GEMINI-1&2, respectively. Using a 10% non-inferiority margin, DTG + 3TC was non-inferior to DTG + TDF/FTC at Week 144 in both GEMINI-1&2 and in the pooled analysis. Response rates across baseline HIV-1 RNA subgroups were high and similar in both arms in the pooled analysis, including in participants with baseline HIV-1 RNA >100,000 c/mL (Table 1). Results were also generally consistent regardless of age, sex or race. While response rates remained lower in DTG + 3TC participants compared with DTG + TDF/FTC participants with CD4+ ≤200 cells/mm^3, differences were smaller than at Weeks 48 and 96; most reasons for non-response were unrelated to virologic efficacy or treatment regimen. Across both studies, 12 participants on DTG + 3TC and 9 on DTG + TDF/FTC met confirmed virologic withdrawal (CVW) criteria through Week 144; none had treatment-emergent TSTi or NRTI resistance mutations. One non-CWV DTG + 3TC participant with reported non-adherence developed M184V at Week 132 and added R263K/R at Week 144, conferring a 1.8-fold change in DTG susceptibility.

Conclusion: In GEMINI-1&2, DTG + 3TC was non-inferior to DTG + TDF/FTC in treatment-naive adults at Week 144, demonstrating durable efficacy. The subgroup efficacy results at Week 144 were generally consistent with overall study results and further demonstrate that DTG + 3TC is an effective initial treatment for HIV-infected patients across a spectrum of disease characteristics and patient populations.

<table>
<thead>
<tr>
<th>Table 1. Proportion of Participants With Plasma HIV-1 RNA &lt;50 c/mL at Week 144 Stratified by Subgroups - ITT-E Population</th>
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<tr>
<td>Overall population</td>
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<td>Adjusted difference (95% CI)</td>
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<td>Age (years)</td>
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<td>Sex</td>
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<td>Baseline HIV-1 RNA (c/mL)</td>
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<tr>
<td>Baseline CD4+ cell count (cells/mm^3)</td>
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<td>Pooled Comparator</td>
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415 4-YEAR OUTCOMES OF B/F/TAF IN TREATMENT-NAIVE ADULTS

Kimberly Workowski1, Chloe Orkin1, Paul Sax1, Debbie Hagins2, Ellen Koenig3, Jeffrey Stephens4, David A. Wohl5, Adriano Lazzarin6, Samir Gupta7, Rima K. Acosta1, Jason Hindman1, Diana Brainard8, Samir Gupta10, Emory University, Atlanta, GA, USA, 2Barts Health NHS Trust, London, UK, 3Bingham and Women’s Hospital, Boston, MA, USA, 4Chatham County Health Department, Savannah, GA, USA, 5Instituto Dominicano de Estudios Virológicos, Santo Domingo, Dominican Republic, 6Mier University, Mason, GA, USA, 7University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 8San Raffaele Hospital Milan, Milan, Italy, 9Indiana University, Indianapolis, IN, USA, 10Gilead Sciences, Inc, Foster City, CA, USA

Background: Bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) is a guidelines-recommended single-tablet regimen for people with HIV-1 (PWH). We present cumulative outcomes from open-label extension (OLE) that followed 144 Weeks (W) of blinded treatment in phase 3 studies in treatment-naive PWH.

Methods: We conducted 2 randomized, double-blind, phase 3 studies of B/F/TAF in treatment-naive adults – Study 1489: B/F/TAF vs abacavir/lamivudine (DTG/ABC/3TC) and Study 1490: B/F/TAF vs DTG+F/TAF. After completing 144W of blinded treatment, participants were offered to continue on B/F/TAF for 96W in the OLE. Efficacy was assessed as the proportion with HIV-1 RNA <50 copies/mL at each visit after starting B/F/TAF using missing=excluded (M=E) analysis; safety by adverse events (AEs) and laboratory results. Bone mineral density (BMD) in BMD was measured in those randomized to B/F/TAF in Study 1489. We present cumulative results for all participants treated with B/F/TAF in the randomized or OLE phases through a maximum of 192 weeks of follow up (i.e. OLE W48). The final phase of this study will complete once all participants reach a total of 240 weeks (i.e. OLE W96).

Results: In Study 1489, 314 participants were randomized to B/F/TAF and 315 to DTG/ABC/3TC; 252 and 254 entered the OLE. In Study 1490, 320 were randomized to B/F/TAF and 325 to DTG+F/TAF; 254 and 265 entered the OLE. Efficacy was >96% after W48 at each study visit through W192 in both studies. The rate of discontinuation during the OLE analysis window. Grade 3 or 4 drug-related AEs was 3.8% in Study 1489 and 3.1% in Study 1490. Abnormal lab values included increases in creatinine (2.4%), decreases in total cholesterol (2.3%) and increases in AST (2.3%) in Study 1489. Across both studies, only 1 participant experienced an AE that led to drug discontinuation during the OLE analysis window. Arm 3 or 4 drug-related AEs were rare (Table). There were no discontinuations due to renal AEs. In participants initially randomized to B/F/TAF, the median change in weight from baseline to W192 was 4.6 kg in Study 1490 and 5.0 kg in Study 1490. The mean percent changes (SD) in hip and spine BMD were W192 were 1.5% (4.9) and -0.9% (5.2), respectively. 13% of participants with baseline osteopenia in hip and 3% with osteopenia were normal at normal baseline hip and 6% with normal baseline spine BMD progressed to osteopenia and none developed osteoporosis.

Conclusion: Over 4 years of follow-up in treatment-naive participants, B/F/TAF was safe and highly efficacious. Similar outcomes were demonstrated in participants who switched from DTG-containing regimens to B/F/TAF. These results confirm long term safety and efficacy of B/F/TAF.

<table>
<thead>
<tr>
<th>Table 1. Proportion of Participants With Plasma HIV-1 RNA &lt;50 c/mL at Week 144 Stratified by Subgroups - ITT-E Population</th>
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<td>Race</td>
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<tr>
<td>Baseline HIV-1 RNA (c/mL)</td>
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<tr>
<td>Baseline CD4+ cell count (cells/mm^3)</td>
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**Note**: Distributions are consistent at W192 for the groups randomized to B/F/TAF and BMD treatment with B/F/TAF vs the OLE for switch groups.
**416 WEEK 96 ANALYSIS OF VIRAL BLIPS FROM A PHASE 2B TRIAL OF ISLATRAVIR AND DORAVIRINE**

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1Queen Mary University of London, London, UK, 2University of Paris Diderot, Paris, France, 3Hospital Bichat Claude Bernard, Paris, France, 4Hospital Herman Hernandez- Aranema de Temuco, Temuco, Chile, 5University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 6Merck & Co, Inc, Kenilworth, NJ, USA

**Background:** Islatravir (ISL, MK-8591) is the first nucleoside reverse transcriptase translocation inhibitor (NRTTI) in development for the treatment and prevention of HIV-1 infection. Previously we showed that ISL+DOR demonstrated efficacy in maintaining viral suppression and was well tolerated through week 96 in a Phase 2b trial. Rates of protocol defined virologic failure (PFV) were low across all groups. Here we report on an analysis of blip frequency—a sensitivity marker for efficacy.

**Methods:** In a Phase 2b trial in treatment-naive adults with HIV-1 participants were randomized to receive ISL (0.25, 0.75 or 2.25 mg) + DOR (100 mg) and lamivudine (3TC, 300 mg) QD, or a fixed-dose combination of DOR, 3TC and tenofovir disoproxil fumarate (DOR/3TC/TDF). Participants receiving ISL achieving HIV-1 RNA <50 copies/mL week 20 or later stopped 3TC at the next study visit to transition to the two-drug regimen for Part 2 of the trial. For this current analysis we analyzed viral blip frequency for participants who entered Part 2 of the trial through week 96. A viral blip was defined as an HIV-1 RNA ≥50 copies/mL value observed between two values of <50 copies/mL after achieving initial response.

**Results:** 114 participants entered Part 2 of the trial and were included in the analysis. During Part 2 of the trial through week 96, a higher percentage of participants on the three-drug regimen in the DOR/3TC/TDF group experienced viral blips as compared to participants on the two-drug regimen in the combined ISL groups; 4 blip episodes occurred in 4 out of 28 participants (14.3%) in the DOR/3TC/TDF group as compared to 8 blip episodes in 7 of 86 participants (8.1%) in the combined ISL groups (Table 1). Of the participants with viral blips, 5 of 7 participants in the combined ISL groups and 0 out of 4 participants in the DOR/3TC/TDF group had baseline HIV-1 RNA >100,000 copies/mL. All participants with viral blips, including those with high baseline HIV-1 RNA levels, were suppressed by the next study visit and remained suppressed through week 96. None of the participants with viral blips had subsequent viral rebound or PFV.

**Conclusion:** Viral blips were relatively rare for all treatment groups and were not associated with a loss of virologic suppression or PFV.

**Table 1:** Frequency of Viral Blips for participants who entered Part 2 of the trial

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Parameter</th>
<th>N=29</th>
<th>N=10</th>
<th>N=27</th>
<th>N=85</th>
<th>N=114</th>
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<tbody>
<tr>
<td>Part 2</td>
<td>Number of Participants with Blips, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 96</td>
<td>3 (10.3)</td>
<td>1 (10.0)</td>
<td>5 (15.1)</td>
<td>7 (8.1)</td>
<td>14 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Number of Distinct Blip Episodes</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>4</td>
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<td></td>
</tr>
</tbody>
</table>

**417 SWITCHING TO DTG/3TC FDC IS NONINFERIOR TO TBR FOR 96 WEEKS: TANGO SUBGROUP ANALYSES**

Paul Benson, Clifford A. Kinder, Maria Jesus Perez-Elias, Don E. Smith, Stefan H. Scholten, Mouini Ait-Khalef, Keith A. Pappa, Ruolan Wang, Jonathan Wright, Brian Wynne, Michael Aboud, Jean A. Van Wyk, Kimberly Smith

1Be Well Medical Center, Berkley, MI, USA, 2AIDS Healthcare Foundation - The Kinder Medical Group, Miami, FL, USA, 3Hospital Universitario Ramón y Cajal, Madrid, Spain, 4Albion Centre, Sydney, Australia, 5Praxis Hohenstaufenring, Germany, 6ViiV Healthcare, Brentford, UK, 7ViiV Healthcare, Research Triangle Park, NC, USA

**Background:** The 2-drug regimen (2DR) of DTG/3TC reduces the number of antiretroviral agents taken by individuals treated for HIV-1 infection, when compared to traditional 3DRs. DTG/3TC is non-inferior to DTG+TDF/FTC in HIV-1 infected ART-naive adults (GEMINI) through Week 144 and in ART-experienced, virologically suppressed participants switching from a TAF-based 3/4DR (TANGO) through Week 96. Here we present a key Week 96 secondary endpoint from the TANGO study: Snapshot virologic success by baseline regimen third agent class, disease and demographic characteristics.

**Methods:** TANGO is a randomized, open-label, multicenter, non-inferior Phase III study evaluating the efficacy and safety of switching to DTG/3TC once daily versus remaining on a current TAF-based regimen in HIV-1 infected adults, with HIV-1 RNA<50/c/mL for >6 months, on a TAF-based regimen for at least 3 months and without prior virologic failure or historical NRTI or INSTI major resistance mutations, were eligible to participate. Randomization was stratified by baseline 3rd agent class: PI, NNRTI, INSTI. The primary endpoint was the proportion of participants with plasma HIV-1 RNA<50/c/mL at Week 48 (FDA Snapshot algorithm, Intention To Treat-Exposed [ITT-E] population) with secondary analyses at Week 96.

**Results:** 741 randomized/exposed participants (DTG/3TC: 369; TBR: 372) were included. Snapshot success rates across subgroups were generally consistent with the overall TANGO Week 96 study results and were similar between arms (Figure). Zero participants on DTG/3TC and 3 participants (<1%) on TBR met confirmed virologic withdrawal criteria with no resistance mutations observed at failure.

**Conclusion:** Switching to DTG/3TC FDC was non-inferior to continuing a TAF-based 3DR in maintaining virologic suppression in HIV-1 infected ART-experienced adults through Week 96. Efficacy by subgroups was consistent with overall Week 96 study results, demonstrating that switching from TAF-based regimens to DTG/3TC is effective at maintaining virologic suppression regardless of baseline regimen, patient or disease characteristics.

**Figure. Proportion of Participants With Plasma HIV-1 RNA<50 c/mL at Week 96: Snapshot Analysis by Subgroups – ITT-E Population**

**418 MISSING DATA, MISSING DIVERSITY: PARTICIPANT DEMOGRAPHICS IN INDUSTRY STUDIES 2010-20**

Liz Barr, Michael J. Donohoe, Murray Penner, Moises Agosto, Danielle Campbell, Bob Huff, Rick Guasco, Andy Kayes, David Palm, for the AIDS Treatment Activists Coalition

1AIDS Treatment Activists Coalition, Denver, CO, USA, 2AIDS Treatment Activists Coalition, Washington, DC, USA

**Background:** Public attention on the need for participant diversity is high, particularly in light of the recent wave of journal and governmental policies requiring studies to report varying elements of participant diversity (sex, race, age) and the FDA's recent decisions to limit certain PrEP indications by sex. AIDS Treatment Activists Coalition (ATAC) is a US-based coalition of AIDS activists who meets regularly with pharmaceutical companies to bring an expert community perspective into the development of new HIV drugs and the utilization of HIV therapies. ATAC undertook a systematic analysis of participant diversity in Industry-sponsored studies for four active companies in HIV research and development (R&D): Gilead Sciences (Gilead), Janssen, Merck, and ViV Healthcare (ViV). The primary objective of this analysis was to characterize participant demographics in efficacy and registrational pharmaceutical
structures (Phase II, III, IV, and Observational studies) from 2010–2020 that were sponsored by these four companies.

Methods: A systematic search of clinicaltrials.gov for any studies related to HIV drugs under development by the four companies during the study time period (2010–2020) was completed. Search results were screened for relevance. Registry listings for studies in final dataset (N=146) were reviewed, and study information (including phase, # of participants, dates, location, and demographics when available) were recorded. Analyses were performed in Excel to characterize trends in participant diversity by company, study phase, study location, and time period.

Results: Participant sex, which was generally reported to clinicaltrials.gov, suggests that male participants are over-represented by 34%. Race-specific data was unreported for 65% of studies, and, when reported, suboptimal. Geographic diversity was lacking, as a majority (75%) of study sites were in the United States.

Conclusion: ATAC recommends that industry: Enroll more cisgender and transgender women, ensuring women participants are representative of the global and local HIV epidemics in race, ethnicity, and age; Enroll participants that reflect the racial and ethnic diversity of PLWHIV — including Black, Hispanic/Latinx, and Native American participants in the United States; Disaggregate data by sex, gender, race, ethnicity, and age. Disaggregate transgender women from MSM in reporting. Replace upper age limits with no upper age limits.

Disaggregate global and local HIV epidemics in race, ethnicity, and age; Enroll participants with the highest unmet needs. Ensure participants in impacted communities.

Background: The efficacy result of 4/7-days strategy was sustained at W96, with a low rate of viral failure, particularly with InSTI based regimen. This 4 consecutive days-on and 3 days-off reduced the cost ART maintenance regimens and represents a real, workable, alternative to the recommended maintenance therapy.

W96 EFFICACY OF 4/7 DAYS MAINTENANCE ART STRATEGY: ANRS-170 QUATUOR TRIAL

Roland Landman1, Lambert Assoumou2, Sidonie Lambert-Nicot1, Jonathan Bellet2, Karine Amat2, Guillette Allavena3, Christine Katlama7, Karine Lacombe2, Jean-Michel Molina5, Roland Landman4

1Institut de Médecine et Épidémiologie Appliquée, Université de Paris, INSERM, IAME, Hôpital Bichat, PARIS, France; 2Sorbonne Université, INSERM, Institut Pierre Louis d’Épidémiologie et de Santé Publique, PARIS, France; 3Sorbonne Université, INSERM, Institut Pierre Louis d’Épidémiologie et de Santé Publique Département de Virologie, Hôpital Saint-Antoine, PARIS, France; 4AP-HIP Hôpitaux Universitaires Pitil-Salpétrière - Charles Foix, Sorbonne Université, INSERM, PARIS, France; 5Hôpital Bichat, PARIS, France; 6Hôpital Hôtel-Dieu, Service des Maladies Infectieuses, Hôpitaux de Paris, France; 7Institut de Médecine et Épidémiologie Appliquée, Hôpital Bichat, PARIS, France; 8Service des Maladies Infectieuses, CHU de Nantes, France; 9Hôpital Pitil- Salpétrière AHPH, Service des Maladies Infectieuses, PARIS, France; 10Hôpital Saint-Antoine AHPH, Service des Maladies Infectieuses, PARIS, France, 11Hôpital Saint-Antoine AHPH, Service des Maladies Infectieuses, PARIS, France, 12Université de Paris, INSERM, IAME, Hôpital Bichat-Claude Bernard, Service des Maladies Infectieuses et Tropicales, AP-HIP PARIS, France, 13Hôpital Raymond Poincaré AHPH, Université Versailles-Saint-Quentin, GARCHES, France; 14Université de Paris, INSERM, IAME, Hôpital Bichat-Claude Bernard, Service des Maladies Infectieuses et Tropicales, AP-HIP PARIS, France, 15Institut de Pharmacie, Hôpital Pitié-Salpétrière, Service de Biochimie et Néphrologie, AHPH, PARIS, France, 16Hôpitaux Universitaires Paris-Ile de France-Ouest, Hôpital Raymond Poincaré AHPH, Université Versailles-Saint-Quentin, GARCHES, France

Background: Intermittent treatment could improve the convenience, tolerability and cost of ART. We have previously demonstrated in the QUATUOR trial the non-inferiority of maintenance 4 days-a-week (4/7days) versus 7/7days in patients (pts) under triple therapy with either PI, NNRTI, or InSTI based regimen: 95.6% vs 97.2% treatment success at W48. ClinicalTrials.gov: NCT02526422. We report here the W96 results.

Methods: Randomized, open-label, multicenter parallel trial evaluating the efficacy and safety of a maintenance 4/7days. Pts with plasma viral load (VL)<50 copies/mL for at least 12 months were randomly assigned in a 1:1 ratio to immediate switch to a 4/7 days (4/7-I) at D0 or to a deferred switch to 4/7 days (4/7-D) at W48. The primary endpoint for the present analysis was the Kaplan-Meier estimated proportion of participants under the 4/7-days strategy (4/7-I group 0–96 weeks and 4/7-D group 48–96 weeks) with treatment success (VL< 50 copies/mL and no treatment strategy modification) at week 96.

Results: Overall, 621 pts on 4/7-days strategy were analyzed (318 in 4/7-I group and 303 in 4/7-D group). The 3rd agent drug class was NNRTI for 28% (46%), InSTI for 30% (48%), and PI for 35% (6%). At W96, therapeutic success with the 4/7-days strategy was 92.6% (95% CI 90.2-95.2) and virological failure (VF, defined as 2 consecutive VL>50 copies/mL) was 4.2% (2.2-6.3%). Of the 318 pts in the 4/7-I group, 14 underwent therapeutic failure including 6 VF until W48 and 11 after W48 (7 VF). Among the 303 pts who switched to 4/7-days strategy at W48, 10 had therapeutic failure (6 VF) after W48. Regarding the 3rd agent class, VF was observed in 5.3% (1.8-8.6) with NNRTI, and 2.4% (0.6-4.1) with InSTI at W96. Overall, among the 19 VF, drug resistance mutations appeared in 7 pts (2 to nucleoside analogs (NAT) alone, 4 to NA and NNRTI, 1 to NA and InSTI (raltegravir). No significant adverse events, biological changes or changes in the level of pro-inflammatory markers were observed with the 4/7-days strategy until W96, except a gain of +4 ml/min (IOR: -2;+6) in eGFR, p<0.001.

Conclusion: The efficacy result of 4/7-days strategy was sustained at W96, with a low rate of viral failure, particularly with InSTI based regimen. This 4 consecutive days-on and 3 days-off reduced the cost ART maintenance regimens and represents a real, workable, alternative to the recommended maintenance therapy.

420 STRUCTURAL BASIS FOR VIRAL RESISTANCE TO LONG-ACTING HIV-1 CAPSID INHIBITOR GS-6207

Stephanie M. Bester1, Reed Haney1, Daniel Adu-Ampatwum2, James Fuchs2, Mamuka Kvaretilashveli1

1University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 2The Ohio State University, Columbus, Ohio, USA

Background: GS-6207 (Lenacapavir, Gilead Sciences) is an experimental long-acting and highly potent HIV-1 capsid (CA) inhibitor. Viral breakthrough assays in cell culture identified a number of HIV-1 CA substitutions including M66I, Q67H, N74D and Q67H/N74D that confer substantial resistance to the inhibitor. Furthermore, the Q67H capsid substitution has emerged in HIV-1 infected patients receiving GS-6207.

Methods: We have determined high-resolution x-ray crystal structures of cross-linked HIV-1 CA hexamers containing the following drug-resistant substitutions: M66I, Q67H, N74D and Q67H/N74D. In addition, we have determined the structures of the CAQ67H and CAQ67H/N74D hexamers containing the following drug-resistant substitutions: M66I, Q67H, N74D and Q67H/N74D. In addition, we have determined the structural basis for how the HIV-1 CA

substitutions confer resistance to the experimental drug GS-6207 and provide the means for rationally developing second-generation inhibitors.

419
421 GSX3640254 IS A NOVEL MATURATION INHIBITOR WITH AN OPTIMIZED VIROLOGY PROFILE
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Myers Squibb, Redwood City, CA, USA
Background: HIV-1 maturation inhibitors (MIs) work late in the replication cycle to block the cleavage of gag p25 into p24 (capsid) and Sp1, resulting in release of noninfectious virions. Previous MIs have demonstrated clinical efficacy but have encountered virologic failures in subjects infected with viruses containing gag polymorphisms such as V362I and the 36-370 region of gag. GSX3640254 (GSX’254) is a new MI with an optimized profile that strongly inhibits viruses containing these polymorphisms.
Methods: A medicinal chemistry approach coupled with a virology triage strategy focused on key gag polymorphisms was used to identify GSX’254. The antiviral activity of GSX’254 against select site-directed mutants (SDMs) was compared to a prior maturation inhibitor, GSX352795 (GSX’795, formerly BMS-986176). Broad spectrum antiviral activity of GSX’254 was also examined against primary isolates across various HIV subtypes and against recombinant viruses with gag genotypes cloned from clinical isolates. In addition, biochemical and resistance studies were used to confirm the mechanism of action (MoA) of GSX’254.
Results: Compared to wild-type virus, SDM viruses with gag changes V362I, V370A, Δ370, or R286K/V370A were equally inhibited by GSX’254. The potency of GSX’254 was greater compared to GSX’795 against a panel of 19 primary isolates (subtype A=3, B=7, C=6, CRF01_AE=3), with a median EC50 of 3 nM (range 1-177 nM) vs. 8 nM for GSX’795 (range 1-1575 nM). Using a panel of 24 Subtype B and 11 Subtype C viruses with broad divergence, GSX’254 exhibited median EC50 values of 1.4 nM (range 0.48-6.9 nM) and 1.4 nM (range 0.85-1.9 nM) for the Subtype B and C viruses, respectively. GSX’254 retained some potency against an A364V SDM (EC50=140 nM) but exhibited a less than optimal maximal percent inhibition (72.5%) and was selected in resistance studies in cell culture. In vitro MoA studies demonstrated that GSX’254 inhibited cleavage of p25 for consensus subtype B gag as well as gag proteins with relevant SDMs.
Conclusion: These data demonstrate the optimized antiviral properties of GSX’254, a once-daily maturation inhibitor, against viruses with common MI-related gag polymorphisms. GSX’254 has been shown to provide significant reduction in viral load in people living with HIV in a phase 2A proof-of-concept study. Together, these data support the ongoing clinical development of GSX’254 in HIV-1 infected individuals.

422 REDUCED SUSCEPTIBILITY TO TEMSAVIR IS NOT LINKED TO IBA OR MVC RESISTANCE
Burt Rose1, Margaret Garland1, Eugene Stewart2, Mark Cocklett1, Peter Ackerman1, Max Lataliade3, Cyril Llamos1, Mark Krystal1
1WV Healthare, Branford, CT, USA, 2WV Healthare, Research Triangle Park, NC, USA, 3GlaussSmithline, Collegeville, PA, USA
Background: Temsavir (TMR), the active agent of the gp120-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor 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To be sensitive by genotyping, 45 (79%), 30 (94%), 23 (92%) and 13 (93%) to EVM by phenotyping. For the 57, 32, 25 and 14 participants predicted out of 96 as sensitive to EVM. Plasma viruses were also evaluated for sensitivity of 75%, 83%, 91%, 97% identified 57, 32, 25 and 14 participants, respectively, for EVM and 3BNC117 sensitivity were identified from a genotypic-phenotypic assay and the phenotypic PhenoSense HIV nAb Assay assessments were carried out following CLIA guidelines as would be required for entry into clinical trials. A positive predictive value (PPV), was calculated for each genotypic location, key population, and self-reported PrEP adherence were collected via national levels, and blood collection kits were distributed. Data on sex, age, other classes of ARVs. Finally, we examined the possibility that Env mutations contribute to drug resistance in vivo.

**Methods:** We propagated clinically relevant HIV-1 strains (subtype B NL(A08) and subtype C, transmitted founder K3016) in the presence of DTG,rilpivirine and emtricitabine to select variants exhibiting resistance to these drugs. Env mutations that arose were introduced into the wild-type (WT) strain and the replication kinetics and cell-free infectivity in the presence of several ARVs targeting RT, IN, PR and Env were examined. We also performed single-genome sequencing of IN/Env-coding regions of plasma-derived viruses from five individuals failing a raltegravir-containing regimen with therapeutic raltegravir levels in ACTG study A5273.

**Results:** By propagating the NL(A08) strain in the presence of DTG, we identified the Env-N654K mutation in gp41 heptad repeat 2 (HR2). We also identified Env-T541Y (gp41 HR1) and Env-E621V (gp41 disulfide loop region) mutations in the K3016 strain in the presence of DTG and RT inhibitors, respectively. These Env mutants exhibit faster-than-WT replication but reduced cell-free infectivity relative to WT and showed reduced sensitivity to DTG (3.6-30 fold), efavirenz (9.5-23 fold) and nevirapin (13-25 fold), but not to T-20, in spreading infection. The Env variants did not alter sensitivity to the ARVs in the context of cell-free infection, suggesting that Env mutations reduce sensitivity to ARVs by enhancing the efficiency of cell-cell transmission. We observed mutations in regions of gp41 analogous to those described above in individuals failing raltegravir-containing ART in the absence of mutations in IN.

**Conclusion:** Our findings suggest that Env-mediated drug resistance may broadly affect HIV sensitivity to ARVs and provide clues towards understanding how ART failure occurs without mutations in drug-target genes.

**EVALUATION OF bNAb SENSITIVITY BY GENOTYPING AND PHENOTYPING FOR HIV CLINICAL TRIALS**

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**Background:** HIV envelope (Env) diversity is a significant challenge for the use of broadly neutralizing antibodies (bNAbs) in HIV treatment and cure studies. Screening Env for bNAb susceptibility to select sensitive participants will be important to improve clinical efficacy, however, no standard approach has been established. Individuals who initiate ART during primary HIV infection generally have low sequence diversity and are an attractive population for early proof of concept bNAb cure-related trials. We therefore analyzed Env sequences from individuals who started ART during primary HIV infection.

**Methods:** Pre-ART plasma virus from 96 participants in the Zurich Primary HIV Infection Study, who initiated ART during primary HIV infection, was genotyped and phenotyped for susceptibility to the bNAbs elipovimab (EVM, formerly GS-9722) and 3BNC117. The genotypic GenoSure HIV Envelope RNA Assay and the phenotypic Phenosense HIV nAb Assay assessments were carried out following CLIA guidelines as would be required for entry into clinical trials. For predicting bNAb susceptibility by genotyping, Env amino acid signatures for EVM and 3BNC117 sensitivity were identified from a genotypic-phenotypic correlation algorithm using a subtype B database (n=203 for EVM, N=234 for 3BNC117). A positive predictive value (PPV), was calculated for each genotypic Env signature based on phenotypic sensitivity to the bNAbs.

**Results:** Bioinformatic methods identified Env signatures with PPsVs from 75% to 97% for EVM, achieving higher PPsVs required more complex Env signatures. Genotyping the plasma virus and applying Env sensitivity signatures with PPsVs of 75%, 83%, 91%, 97% identified 57, 32, 25 and 14 participants, respectively, out of 96 as sensitive to EVM. Plasma viruses were also evaluated for sensitivity to EVM by phenotyping. For the 57, 32, 25 and 14 participants predicted to be sensitive by genotyping, 45 (79%), 30 (94%), 23 (92%) and 13 (93%) participants, respectively, were confirmed sensitive to EVM by phenotyping. Similar analyses were performed for 3BNC117.

**Conclusion:** The genotypic assessment using the developed Env signatures for sensitivity appears as predictive as the direct measurement of sensitivity by phenotyping and may therefore be preferred due to turnaround time and assay simplicity. A significant number of the analyzed participants had Env sequences that are susceptible to EVM and 3BNC117 and could thus be potential candidates for trials involving these bNAbs.

**VARIANT SELECTION, CHARACTERIZATION, AND IMPACT ON ANTIBODY SARS-CoV-2 NEUTRALIZATION**

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**Background:** Monitoring genomic variation of SARS-CoV-2 is crucial in mitigating adaptation to the human host and developing effective treatments that safeguard global health. Bamlanivimab and etesevimab are monoclonal antibodies (mAbs) that have demonstrated potent SARS-CoV-2 neutralizing activity in both pre-clinical and clinical settings and have distinct but overlapping binding sites. Here, the selection and characterization of variants in a pre-clinical setting is presented alongside the impact of emerging variants on antibody binding affinity and viral neutralization potency.

**Methods:** Variant selection was carried out via directed evolution of the receptor binding domain (RBD) and serum passage of authentic SARS-CoV-2 in the presence of bamlanivimab and etesevimab individually or in combination. Sequence confirmed, putative-resistance variants identified in both selection methodologies were incorporated into different assay platforms (VSV-based SARS-CoV-2 pseudovirus neutralization, a yeast RBD display hACE2 competition, and binding affinity to mAb and hACE2) to evaluate potency loss of the selecting mAb and test activity against the mAb combination.

**Results:** Serial passage of SARS-CoV-2 and directed evolution of the RBD protein were unable to select for resistant viral variants under the pressure of mAb combination therapy. In the same experimental paradigm, variants were identified when each mAb was evaluated alone (E484D/K/Q, F490S, Q493R, and S494P for bamlanivimab and K417N, D420N and N460K/S/T/Y for etesevimab). Neutralization and binding assessments confirmed reduced susceptibility of the variants to the single-selecting mAb with 50-fold or greater reductions in potency. Importantly, aside from the Q493R variant, all other resistant viruses were neutralized by the mAb combination therapy.

**Conclusion:** In vitro selection studies using single mAbs, bamlanivimab or etesevimab, identified key positions within the SARS-CoV-2-S protein that have potential for viral resistance in the clinic, whereas similar studies with the mAb combination therapy were unable to select variants. Binding and competition assays confirmed the neutralization phenotyping data and indicates the mechanism of resistance is due to a reduction in binding affinity. The pre-clinical selection and functional characterization of resistant viral variants directly supports the observation that mAb combination therapy results in a lower frequency of treatment-emergent resistance in clinical treatment studies.

**MONITORING OF HIV DRUG RESISTANCE AMONG SEROCONVERTERS ON PrEP IN KENYA**

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**Background:** In 2018, Kenya was the first sub-Saharan African country to nationally implement TDF/FTC PrEP as part of combination prevention in individuals at substantial ongoing risk of HIV infection. Despite high effectiveness, individuals who fail PrEP may risk select resistance to TDF and/or FTC, also used as first-line ART in Kenya. To address this concern, NASCAP and the USAID/PEPFAR-funded GEMS project implemented PrEP resistance monitoring at ~1500 PrEP sites in Kenya and assessed the frequency of HIV drug resistance (HIVDR) mutations among individuals seroconverting after initiating PrEP.

**Methods:** HIVDR monitoring for PrEP seroconverters was implemented through a national protocol that enrolled consenting clients who had access to PrEP. HIVDR content was added to PrEP service provider trainings at county and national levels, and blood collection kits were distributed. Data on sex, age, location, key population, and self-reported PrEP adherence were collected via
questioning. Blood samples were assessed for resistance using population genotyping at WHO-accredited HIVDR laboratory in Kenya. Resistance mutations were identified using the Stanford HIV Drug Resistance Database.

**Results:** Over 2000 service providers were trained and 340 collection kits distributed nationally. From an estimated 25,000 individuals currently on PrEP, 67 seroconversions were reported, and samples were collected from 55 (82%) clients; of whom 40 (73%) were female, with a median age of 30.5 years (18, 67), and included discordant couples (56%), female sex workers (11%), and men who have sex with men (9%). Eleven (20%) seroconversions occurred within 6 weeks of PrEP initiation; 30 (54%) self-reported adherence as “good” (6-7 PrEP doses/week); and 21 (38%) had HIV-1 RNA <1000 c/mL. Of the 30 successfully genotyped samples, 10 (33%) had major HIVDR mutations detected; precisely, non had K65R or K70E, 5 (17%) had M184V, and 9 (30%) had one or more major NNRTI mutation including K103, K103N, V106I, and Y181C.

**Conclusion:** PrEP rollout is highly successful in Kenya, though the number of seroconverters may be underreported despite widespread training and kit dissemination. The rate of PrEP-related resistance with M184V (17%) highlights the importance of continued monitoring for HIVDR in PrEP seroconverters to preserve ART options for both treatment and prevention. Additionally, the increased NNRTI resistance suggests transmitted DR warranting continuous monitoring for pretreatment HIVDR among newly infected persons.

### UltraSensitive HIV-1 Drug-Resistance Analysis in the DISCOVER PrEP TRIAL

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**Background:** The DISCOVER study is an ongoing randomized, double-blind study of pre-exposure prophylaxis (PrEP) using daily FTC/TAF (Descovy; DVF) or FTC/TDF (F/TDF; Truvada; TVD) in men and transgender women who have sex with men. Of the 5,335 randomized participants evaluated for HIV-1 infection, 27 participants (0.5%) became infected with HIV-1 through 144 weeks of study. Participants who acquired HIV were evaluated with population, standard next generation sequencing (NGS), and ultra-sensitive sequencing and the overall resistance data and analysis are presented here.

**Methods:** Plasma samples from participants who become infected with HIV-1 and had a viral load of >400 copies/mL were tested with the GenoSure™ MG assay (Monogram) to analyze the protease (PR) and reverse transcriptase (RT) genes for resistance associated mutations (RAMs) (at ≥15-20% of the viral population). Additional analysis of the PR-RT and integrase (IN) genes on all available plasma samples was performed by standard NGS (SeqIT) to analyze RT codons 63-131 and 152-211 (University of Pittsburgh).

**Results:** By population sequencing, 4/22 participants infected with HIV had M184V, all in the F/TDF group and all with suspected baseline infection; 2 of these 4 also had M184I present. By standard NGS and UMI-NGS, 26/27 HIV participants infected with HIV had samples available and 25/27 were successfully analyzed. For the 4 participating F/TDF with M184V, each had M184V also detected. By UMI-NGS, 1 participant on F/TDF had the M184V mutation present at 2%. Ten participants had additional mutations conferring resistance to non-drugs including InSTI RAMs T66A, E92G, Y143C, Q148R, N155H; PI RAMs M46I; NNRTI RAMs V90I, V106I, K103N, Y188L, which were presumed to be transmitted.

**Conclusion:** Using population sequencing and standard NGS, M184V was detected in 4 participants, all in the F/TDF arm. With ultra-sensitive UMI-NGS testing, similar results were observed in the F/TDF arm, with the addition of 1 participant with M184V in the F/TDF arm. In addition, analysis by standard NGS of the PR, RT and IN genes found notable transmitted drug resistance to non-study drugs. Overall, resistance to study drugs in the DISCOVER study was infrequently seen and primarily with suspected baseline infections.
430 HIV WITH TRANSMITTED DRUG RESISTANCE IS DURABLY SUPPRESSED BY B/F/TAF AT WEEK 144
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Background: Two phase 3, randomized, double-blind, active-controlled studies of initial HIV-1 treatment demonstrated that bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) was non-inferior to dolutegravir/abacavir/lamivudine (DTG/ABC/3TC, Study 1489) or to DTG+F/TAF (Study 1490) through 144 weeks. In both studies, there was no emergent resistance to study drugs. Here, we describe the effect of baseline transmitted drug resistance (TDR) on treatment response over 3 years.

Methods: Population sequencing of HIV-1 protease and reverse transcriptase (RT) was performed at screening; resistance to study nucleos(t)ide reverse transcriptase inhibitors (NRTIs) was excluded. Retrospective baseline next generation sequencing of protease, RT, and integrase (IN) was analyzed at a ≥15% cutoff. Treatment outcomes were assessed at Week 144 using last on-treatment observation carried forward (LOCF). Resistance analyses were performed on participants with confirmed viral rebound of HIV-1 RNA ≥200 copies/mL while on study drug.

Results: Of 1421 PLWH screened for both studies, only 3 (0.2%) were excluded due to TDR to FTC, TAF, ABC, or 3TC. TDR was present in 19.5% (248/1274) of enrolled participants. Among persons with low-level viremia while taking NNRTI-based ART, 17/21 (81%) control arm participants harboured HIV with resistance to ≥2 drugs (NNRTI + NRTI + PI), while no PI-associated resistance was detected in the five switch (second-line) participants. At baseline, 31/37 (84%) participants harboured HIV with nucleoside reverse transcriptase (NRTI) and NNRTI resistance (at one or both time points) was 41/49 (84%) and 42/49 (86%), respectively. Considering each time point individually, sequencing was successful for 37/80 (46%) participants at baseline and 26/48 (54%) participants without viral suppression to <50 copies/mL at 36 weeks (21 control; 5 switch). At baseline, 31/80 (39%) participants harboured HIV with high-level resistance to ≥2 drugs of their current (first-line) regimen, while no PI-associated resistance was detected in the five switch (second-line) participants.

Conclusion: Among persons with low-level viremia while taking NNRTI-based first-line ART enrolled in the SESOTHO trial, the majority harboured HIV-1 with preexisting resistance with or without TDR were comparable (98% of those with primary TDR had HIV RNA <50 copies/mL vs. 97% of those without TDR) (Table), indicating that preexisting TDR did not affect treatment outcomes. One participant had preexisting Q148H+G140S in IN and K70R and K103N in RT at baseline. This participant was randomized to B/F/TAF, had HIV RNA <50 copies/mL at Week 4, and maintained HIV RNA <50 copies/mL through Week 144. In total, 21 participants qualified for post-baseline resistance testing (1.3% [8/634] B/F/TAF, 1.9% [6/315] DTG/ABC/3TC, 2.2% [7/325] DTG+F/TAF); of those, 2/8 B/F/TAF, 6/6 DTG/ABC/3TC, and 4/7 DTG+F/TAF participants had multiple confirmed virologic rebounds during the studies. No participant had emergent resistance to study drugs.

Conclusion: Initial HIV-1 treatment with B/F/TAF, DTG/ABC/3TC, or DTG+F/TAF achieved high, durable rates of virologic suppression. The presence of TDR did not affect treatment outcomes, and there was no treatment-emergent resistance through 144 weeks.
432 EVALUATION OF COMBINATIONS OF CLINICAL MUTAGENIC RESISTANCE ON
InSTI RESISTANCE
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Background: While major integrase strand transfer inhibitor (InSTI) resistance mutations have been identified, the effect of mutation combinations on phenotypic resistance is less clear. We identified a clinical HIV phenotype sequence with four major integrase resistance mutations, and characterized in vitro InSTI phenotypic susceptibility of all combinations thereof to deconstruct their individual and combined effects.

Methods: Routine clinical testing identified an integrase sequence harboring T97A, E138K, G140S and Q148H. We constructed chimeric NL4-3 viruses harboring i) all 15 combinations of these mutations in the autologous integrase backbone, ii) the autologous sequence with these four sites “reverted” to consensus B residues and iii) NL4-3 with all four mutations. Chimeric viruses were grown in a reporter CD4+ T-cell line in the presence of 0.01-1,000nM raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG), cabotegravir (CAB), and bictegravir (BIC), where infection was measured by imaging cytometry.

Results: Consistent with the known fitness impact of Q148H and its compensation by G140S, viruses engineered with Q148H without G140S either failed to propagate, or propagated only after in vitro mutation; these were excluded from analysis. In the autologous viral backbone, T97A, E138K, or G140S alone conferred 2.4- to 15.4-fold decreased susceptibility to EVG but not to other InSTIs (Table 1). Two-mutation combinations conferred low, moderate, except G140S/Q148H which eliminated RAL and EVG activity and conferred 8.3-, 50.9-, and 3.1-fold reduced susceptibility to DTG, CAB, and BIC respectively. Addition of E138K to G140S/Q148H conferred 12.1, 98.6 and 4.6-fold less susceptibility to DTG, CAB, and BIC respectively, while addition of T97A to G140S/Q148H conferred >100-fold resistance to all InSTIs. The quadruple NL4-3 mutant displayed >100-fold less susceptibility to RAL, EVG and CAB but only 66.2-, and 8.2-fold less to DTG, and BIC, respectively, while the clinical revertant retained 2.8-fold decreased susceptibility to EVG. Together this suggests that the autologous clinical backbone also contributed to resistance. Measured EC50 correlated strongly with Stanford HIVdB resistance scores (Spearman r=0.87, p<0.0001 for all InSTIs).

Conclusion: High-level resistance to DTG, CAB and BIC requires multiple integrase substitutions including compensatory mutations.

434 ANALYSIS OF INTRAHOST Gag-Pol EVOLUTION WITH NOVEL SINGLE-
MOLECULE SEQUENCING METHOD
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Background: Technical challenges remain in the sequencing of circulating HIV RNA due to its high intrahost diversity. This bottleneck is particularly pronounced when interrogating long-range co-evolution, which has hampered the direct observation of genetic interactions that code for protein-protein interfaces with relevance in drug and vaccine development. To overcome the read-length limitations of NGS and the problematic error rates of long-read sequencing platforms, we developed MrHAMER, a nanopore-based long-read viral sequencing pipeline that yields thousands of accurate Gag-Pol sequences from individual circulating virions in clinical samples.

Methods: MrHAMER uses concatenated sense/antisense repeats of each 5kb Gag-Pol cDNA (derived from single RNA molecules) to reduce Nanopore long-read sequencing error from 10% to 0.1%. This end-to-end pipeline is extensively validated for HIV RNA sequencing of clinical samples containing low input amounts (as few as 2,500 copies/mL). This includes development of a novel emulsion-based series of PCR-reactions that we determined are critical to prevent artefactual template switching and without which long-range epistatic interactions cannot be resolved.

Results: We use MrHAMER to precisely follow synonymous and non-synonymous changes in the HIV-1 Gag-Pol region (~5 kb) of individual viruses within a single host before treatment initiation and after failure of cART. We detect a hard selective sweep of a single pre-existing Gag-Pol variant containing 25 linked mutations from 0.1% enrichment prior to treatment initiation, to greater than 50% enrichment during antiviral therapy failure. These linked mutations are evenly spread throughout the Gag-Pol ORF, with 44% being synonymous, and 56% resulting in amino acid changes (including one canonical primary drug-resistance mutation that was previously undetected via clinical testing). The enrichment of a high number of linked mutations (instead of more energetically favorable reversions) implies accumulated mutations play a compensatory role in viral fitness.

Conclusion: MrHAMER can identify long-range genetic correlates of intrahost viral evolution in response to antiviral therapy and immune pressure, and enable the identification of novel host-viral and viral-viral interfaces that play a role in viral pathogenesis and can be modulated for therapeutic benefit.

433 PUBLIC AVAILABILITY OF HIV POL SEQUENCES AND ART HISTORIES IN
ACQUIRED HIVDR STUDIES
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Background: The public availability of HIV-1 pol sequences from PWH with viral failure (VF) on ART make it possible to fully define the epidemiology of acquired HIV drug resistance (HIVDR), and the extent of ART cross-resistance associated with specific ART regimens. We therefore sought to determine how often the sequences and linked ART histories from published papers of acquired HIVDR are made publicly available.

Methods: We performed a systematic review of studies in PubMed since 2010 describing pol sequences from ≥25 adult PWH with VF on ART. Studies with median sequence year was before 2007 were excluded. Studies of previously PI-naive PWH receiving atazanavir or darunavir/r, previously NNRTI-naive PWH receiving rilpiravir or doravirine, and previously InSTI-naive persons receiving dolutegravir were included even if they included fewer than 25 PWH.

Results: 351 published studies met inclusion criteria including (i) 124 studies of WHO 1st-line NNRTI (NVP/EFV)-containing regimen; (ii) 53 studies of a PI-containing regimen; (iii) 7 studies of a 2nd-generation NNRTI; (iv) 32 studies of an InSTI-containing regimen; (v) 20 studies containing mixtures of PWH receiving the preceding types of ART; and (vi) 115 studies of uncertain or complicated ART regimens not conforming to the preceding categories. Sequences from 163 studies (46.6%) were publicly available in GenBank, and 71 (20.2%) had linked ART histories in the Stanford HIV Drug Resistance Database. Of 45 clinical trials, sequences were available for 11 (24.4%) and sequences plus linked ART histories were available for 6 (13.3%). Among the 18 journals publishing ≥10 studies, the proportions of studies with publicly available sequences ranged from 10% to 87%. There was no significant temporal increase in the proportion of studies with publicly available sequences (OR: 0.98; 95% CI: 0.93-1.03; p=0.4).

Conclusion: Among 351 recently published studies of sequence data from ART-experienced PWH, sequences were available for less than half of the studies and less than a quarter had linked sequences plus ART histories available. Further increases in data sharing are required to fully define the genetic correlates and epidemiology of acquired HIVDR globally. This information in return is critical to the development of ART guidelines in regions where resistance testing is not routinely available.
A MULTIPLEXED HIV DRUG RESISTANCE (DR) ASSAY TO SURVEY HIVDR MUTATIONS IN POL REGION


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Background: As the WHO universal treatment policy using dolutegravir (DTG) for HIV treatment approaches full implementation globally, a user-friendly, sensitive, low-cost HIVDR assay to survey Integrase (INT) HIVDR is urgently needed. By incorporating newly designed INT primers into Thermo Fisher (TF) HIV-1 Genotyping Assay, we constructed a multiplexed assay to detect HIVDR mutations of Protease, Reverse-transcriptase (PRRT), and INT in pol region, and evaluated assay performance.

Methods: The assay is designed to have a multiplexed reverse-transcription step to generate two amplicons (PRRT and INT) at the same time. Each amplicon is then further amplified and sequenced by Sanger sequencing individually for HIVDR detection. All sequences were analyzed by Stanford HIVdb program. A total of 190 clinical and analytical plasma and dried blood spots (DBS) samples from international HIVDR External Quality Assessment programs, INT clones, and commercial sources were used to evaluate subtype coverage, accuracy, assay sensitivity, precision, and reproducibility. RNA was extracted from 200 µL of plasma or one DBS spot by NucliSens (bioMérieux). RNA of 10 µL per sample was then processed using the assay. We assessed subtype coverage by evaluating 139 samples with previously known subtypes. We evaluated accuracy by testing 86 (INT) and 90 (PRRT) plasma and DBS samples that had reference sequences available. Assay sensitivity was evaluated by amplification success rate of samples with viral load (VL) from 1000 to 5000, and >5000 copies/mL. Precision and reproducibility were evaluated by 436 SIMULTANEOUS HIV QUANTIFICATION AND HIVDR DETECTION BY A SEMICONDUCTOR BIOCHIP SYSTEM

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Background: In regions that do not perform routine HIVDR testing, care providers are often uncertain of how to manage PWI because it is not possible to distinguish PWI who have HIVDR and require an ART change from those remaining infected with wildtype viruses.

Methods: We used a novel semiconductor biochip with a closed-tube near point-of-care NAAT to simultaneously measure VL and detect DRMs at 6 RT (65, 103, 106, 181, 184, 190) and 3 integrate (148, 155, 263) DRM positions.

Results: We performed 4 sets of experiments using a variety of DNA templates containing wildtype and mutant variants at each DRM position. The third set showed that probes complementary to the limiting primers yielded reproducible cycle thresholds (Cts) inversely proportional and linearly related to log DNA copy number. For 4 of 6 probes, the Ct difference between 50 copies and the no template control (NTC) sample was ≥2 cycles and between 100 copies and the NTC sample was ≥3 cycles suggesting that 50 and 100 copies could be unambiguously distinguished from the NTC. The 2nd set showed that melt-curve analysis reproducibly identified the correct codon at 9 DRM positions in mixtures of wildtype and mutant templates ranging from 20%-100%. The 3rd set showed that even at low template concentrations, asymmetric multiplex PCR generated sufficient ssDNA for melt-curve analysis for all probes with one exception. The 4th set showed that the use of additional probes with variant flanking sequences increased the proportion of samples that could be genotyped despite sample sequence variability.

Conclusion: Further development of this biochip system into a mass-deployable point-of-care test would streamline ART delivery and increase the likelihood that PWI would maintain virological suppression and be retained in care.

Table 1. Amplification Sensitivity

<table>
<thead>
<tr>
<th>VL copies/mL</th>
<th>Sample Type</th>
<th># of Samples</th>
<th>PRRT PCR Rate (%)</th>
<th>INT PCR Rate (%)</th>
<th>Sample Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5000</td>
<td>ODS</td>
<td>23</td>
<td>95.7 (±3.0)</td>
<td>95.3 (±2.9)</td>
<td>100/R</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>28</td>
<td>97.8 (±5.0)</td>
<td>97.8 (±5.0)</td>
<td>100/R</td>
</tr>
<tr>
<td>1000 to 5000</td>
<td>ODS</td>
<td>25</td>
<td>98.8 (±2.0)</td>
<td>98.8 (±2.0)</td>
<td>100/R</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>28</td>
<td>98.8 (±2.0)</td>
<td>98.8 (±2.0)</td>
<td>100/R</td>
</tr>
</tbody>
</table>

DR MUTATIONS IN HIV PROVIRUS ARE ASSOCIATED WITH HYPERMUTATIONS

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Background: HIV plasma virus drug resistance mutations (DRMs) tests are crucial to clinical care. However, current available methods require the plasma RNA copy number to be at least 500-1000 copies/ml and can only detect the major viral quasispecies in peripheral blood. HIV proviral sequencing overcomes the limit of plasma viral load requirement by detecting all the “archive mutations”, but its clinical relevance remains to be evaluated.
Methods: We included 25 participants from AIDS Clinical Trials Group (A371, A5068, A5197, A5170, and A5024) with available proviral and plasma viral sequences (either near full length or Pol sequences) and used the genotypic sensitivity score (GSS) to evaluate the level of resistance in their provirus and plasma virus. Defective sequences were further categorized as sequences with and without hypermutations. Personalized GSS score (pGSS, maximum value 3, indicating virus sensitive to current three ARTs) and total GSS score (tGSS, maximum value 15, indicating virus sensitive to a panel of 15 ARTs) were calculated using Stanford University HIV Drug Resistance Database to evaluate the level of resistance to a whole panel of ARTs and to certain ARTs that a participant was using. The rate of sequences with DRMs within each sequence compartment (intact, defective and plasma viral sequences) was calculated for each participant.

Results: Defective proviral sequences were less sensitive than intact proviral sequences or plasma sequences to a panel of 15 antiretroviral therapies (ART) and each participant’s current ART, as reflected by significantly lower pGSS and tGSS (Figure A-B). They harbored more DRMs than other sequence compartments, with a median DRM rate of 0.25 compared to intact sequences (0.0, P=0.014) and plasma sequences (0.095, P=0.30) (Figure C). Hypermutated defective sequences were the major source of DRMs, with a median DRM rate of 1.0 compared to defective sequences without hypermutations (0.042, P<0.001, Figure D-F). Certain Apolipoprotein B Editing Complex 3 (APOBEC)-related DRMs including reverse transcriptase gene mutations M184I, E138K, E138Q, G185A, G185D, G185E, M230L, G190E and protease gene mutations M46I, D30N were enriched in hypermutated sequences but not in intact sequences or plasma sequences. The majority (>95%) of hypermutated sequences had premature stop codons due to APOBEC3.

Conclusion: Provirial sequencing may overestimate DRMs as a result of hypermutations. Removing hypermutated sequences is essential in the interpretation of proviral drug resistance testing.

Results: For 90 blood draws from 70 participants (79% male; age 56 y, 714 CD4 count, 86% subtype B), 257 genotype reports were analyzed. Overall, reproducibility was similar for all PR, RT and IN mutations (86±25%, 86±25% and 87±25%, respectively). A total of 15 PI, 18 NRTI and 19 NNRTI primary DRMs were detected in 21, 31, and 31 participants, respectively, with reproducibility of 84±27%, 83±26%, 78±28% (Fig). In NRTI-R occurrence was too low (n=2) for further analyses. The NRTI DRM M184V had a reproducibility of 82±25%, with 14% of cases being detected in 1/4 or 1/3 reports, 23% in 1/2, 1/4, or 2/3 reports, and 63% being detected in all reports. Reproducibility did not differ among drug classes. Reproducibility of polymorphisms and other non-R RT mutations was significantly higher than for primary NRTI and NNRTI DRMs (p<0.05). Reproducibility of primary DRMs was 10-16% higher when detected by historical genotype compared to not being reported or not having data (p<0.05). By modelling, if a person had a PI, NRTI, or NNRTI DRM, the probability of it being reported by the assay was 76-80%.

Conclusion: Mean reproducibility of ~80% with standard deviations of ~25% indicate that detection of mutations is variable. DNA genotyping may aid clinicians when switching HIV regimens, but these data reinforce the need to interpret tests with caution, as not all mutations may be reported.

438 HIV-1 DNA GENOTYPING IS OFTEN VARIABLE IN REPEAT TESTING FROM SINGLE BLOOD DRAWS

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Background: HIV-1 DNA genotyping assesses archived drug resistance mutations (DRMs) in individuals with low plasma HIV RNA; however, assays detecting these mutations are insensitive. Here we seek to characterize the variability of DNA genotyping by quantifying the reproducibility of mutation reporting from multiple assays from a single blood draw.

Methods: DNA genotyping of protease (PR), reverse transcriptase (RT) and integrase (IN) used GenoSure Archive2 (Monogram Biosciences, CA, USA) from whole blood from suppressed participants with documented resistance from 3 clinical trials (NCT03631732; NCT03110380; NCT03405935). Multiple tests (2-4) were run from each whole blood sample. Reproducibility of primary PR inhibitor (PI)-resistance (R), nucleos(t)ide RT inhibitor (NRTI)-R, non-NRTI (NNRTI)-R, IN strand transfer inhibitor (INSTI)-R, and other non-R mutations were calculated as the number (N) of times the mutation was detected/N of assays run (%). Means ± standard deviations (SD) were reported, and comparisons used Wilcoxon Rank Sum tests. A zero-truncated binomial model was used to estimate the probability of mutation detection.

439 REINFECUTION WITH THE HEPATITIS C VIRUS IN MEN WHO HAVE SEX WITH MEN

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Background: Reinfection with the hepatitis C virus (HCV) after cure has been identified as a major challenge for HCV micro-elimination targets in high-risk groups. In men who have sex with men (MSM), even multiple HCV reinfections have been described, and a specific risk behavior pattern may have a significant impact on management and prevention. Here, we assess factors associated with HCV reinfection(s) among MSM in Germany.

Methods: The German NoCo cohort consists of patients from six German HIV and hepatitis treatment sites providing care for more than 8000 HIV-positive MSM and serving as primary care providers for HIV- MSM and HIV pre-exposure prophylaxis (PrEP) sites. Virologic data, HCV treatment data, risk factors, and behavior as well as liver disease assessment are assessed regularly. In this analysis, patients who were diagnosed with recently acquired HCV reinfection since 2014 were evaluated as a subgroup and compared to patients with a single HCV infection.

Results: Between January 2014 and September 2020, 81/214 (37.8%) men with recently acquired HCV reinfection were included, and during a follow-up time of 416 person-years (py) the incidence rate for HCV reinfection was 18.5/100 py (95% confidence interval (CI) 14.6 – 23.1). 75 subjects had complete datasets: HCV reinfection occurred after treatment-induced cure in 60 (80%) and after spontaneous clearance in 13 (17.3%) cases. Only two reinfections occurred in HIV-negative individuals. Most reinfections were detected through routine HCV RNA testing (68%), followed by testing for ALT elevation (25.6%). Compared to patients with primary HCV infection, reinfection cases were older (OR 1.06, p<0.05). Compared to patients with a single reinfection (n=58) patients with multiple reinfections (n=23) were not significantly different with regards to demographics, STD history, mode of transmission, or substance use.
Conclusion: In the German NoCo cohort, HCV reinfection is frequent, especially in an aging HIV coinfected population with methamphetamine use. The role of recreational ketamine in this setting needs further study, as well as the (so far) infrequent detection of HCV reinfection in the HIV-negative MSM population. No specific pattern could be identified for patients with multiple HCV reinfections.

440 COST-EFFECTIVENESS OF HCV TESTING STRATEGIES FOR HCV ELIMINATION AMONG MSM IN THE US

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Background: Despite current hepatitis C virus (HCV) elimination efforts in the United States (US), men who have sex with men (MSM), both with HIV and without HIV, continue to have high rates of HCV transmission. We hypothesized that better testing could result in a lower rate of transmission. We therefore used a cost-effectiveness model to determine the optimal HCV testing strategies for HCV elimination among MSM.

Methods: We adapted a cost-effectiveness model of HIV and HCV transmission among MSM to determine the cost-effectiveness of improving HCV testing strategies among MSM in the US. The model assumed 15% HCV prevalence among MSM, 8% HCV prevalence among MSM with HIV, and 25% PrEP coverage among MSM without HIV. We evaluated testing strategies that could achieve a 90% reduction in HCV incidence from 2015 as a baseline, through 2030. At baseline, we assumed no systematic HCV screening (i.e. testing only for symptoms) in MSM without HIV not using PrEP (PrEP non-users) and the currently recommended frequency of HCV screening among MSM with HIV (~50%/year). We assessed the following HCV case-finding strategies: screening in parallel with HIV testing in PrEP non-users; screening every 12/6/3 months in MSM using PrEP; and screening every 6 months in MSM with HIV. These strategies were considered alone and in combination, with the cost-effectiveness compared incrementally. Costs (USD) and quality adjusted life-years (QALYs) were assessed to estimate the mean incremental cost-effectiveness ratio (ICER) through 2030, compared to a willingness-to-pay (WTP) threshold of $100,000/QALY gained.

Results: Our economic model predicted the optimal HCV testing strategy to achieve HCV elimination among MSM in the US to be every 6 months for MSM with HIV; annually for MSM using PrEP; and at the time of HIV testing for MSM with HIV. These strategies were considered alone and in combination, with the cost-effectiveness compared incrementally. Costs (USD) and quality adjusted life-years (QALYs) were assessed to estimate the mean incremental cost-effectiveness ratio (ICER) through 2030, compared to a willingness-to-pay (WTP) threshold of $100,000/QALY gained.

Methods: Methods: We adapted a cost-effectiveness model of HIV and HCV transmission among MSM to determine the cost-effectiveness of improving HCV testing strategies among MSM in the US. The model assumed 15% HCV prevalence among MSM, 8% HCV prevalence among MSM with HIV, and 25% PrEP coverage among MSM without HIV. We evaluated testing strategies that could achieve a 90% reduction in HCV incidence from 2015 as a baseline, through 2030. At baseline, we assumed no systematic HCV screening (i.e. testing only for symptoms) in MSM without HIV not using PrEP (PrEP non-users) and the currently recommended frequency of HCV screening among MSM with HIV (~50%/year). We assessed the following HCV case-finding strategies: screening in parallel with HIV testing in PrEP non-users; screening every 12/6/3 months in MSM using PrEP; and screening every 6 months in MSM with HIV. These strategies were considered alone and in combination, with the cost-effectiveness compared incrementally. Costs (USD) and quality adjusted life-years (QALYs) were assessed to estimate the mean incremental cost-effectiveness ratio (ICER) through 2030, compared to a willingness-to-pay (WTP) threshold of $100,000/QALY gained.

Results: Results: Our economic model predicted the optimal HCV testing strategy to achieve HCV elimination among MSM in the US to be every 6 months for MSM with HIV; annually for MSM using PrEP; and at the time of HIV testing for PrEP non-users. This testing schedule resulted in an incremental ICER of $35,000/QALY gained (Table 1).

Conclusion: Conclusions: HCV elimination can be achieved among MSM in U.S. by a relatively nominal and logistically feasible increase in the frequency of HCV screening over what is currently recommended, and is cost-effective.

Table 1. Cost-effectiveness of HCV testing strategies among MSM. An incremental analysis is performed, excluding dominated or weakly dominated strategies.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Baseline</td>
<td>1,576</td>
<td>1,056</td>
<td>1,576</td>
<td>1,056</td>
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<tr>
<td>PrEP users screened every 12 months in parallel with HIV testing</td>
<td>1,576</td>
<td>1,056</td>
<td>1,576</td>
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<tr>
<td>PrEP users screened every 6 months in parallel with HIV testing</td>
<td>1,576</td>
<td>1,056</td>
<td>1,576</td>
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<tr>
<td>PrEP users screened every 3 months in parallel with HIV testing</td>
<td>1,576</td>
<td>1,056</td>
<td>1,576</td>
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</table>

441 HIV/HCV COINFECTION TRENDS IN SPAIN (2015-2019)

Chiara Fanciulli1, Juan Berenguer1, Carmen Busca Arenzana2, Maria Jesus Vivanco1, Maria Jesus Teller2, Lourdes Dominguez1, Pere Domingo3, Jordi Navarro4, Jesus Santos5, Jose A. Iribarren6, Luis Marano7, Marta De Miguel8, Inmaculada Jarrin9, Juan Gonzalez-Garcia10, for the GeSIDA 8514 Study Group

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Background: We assessed the prevalence of anti-HCV antibodies (HCV-Ab) and active HCV infection (HCV-RNA-positive) in people living with HIV (PLWH) in Spain in 2019 and compared the results with four similar studies performed yearly from 2015 to 2018.

Methods: The study was performed in 41 centers (October-November, 2019). The sample size was estimated for an accuracy of 1.0%, the number of patients from each hospital was determined by proportional allocation, and patients were selected using simple random sampling. All oral DAA-based therapy has been available in Spain since the third trimester of 2014. Since June 2017, free access to treatment has been available to all HCV-infected individuals.

Results: The reference population comprised 41,973 PLWH, and the sample size was 1,325. HCV serostatus was known in 1,316 (99.3%), and 376 (28.6%) were HCV-Ab-positive (78.7% PWID and 7.7% MSM). Of the 376 HCV-Ab-positive patients, 291 cleared HCV after anti-HCV therapy, 55 cleared HCV spontaneously, 29 were HCV-RNA-positive, and 1 had unknown HCV-RNA. The prevalence of HCV-RNA-positive was, therefore, 2.2%. As 11 of 29 patients were receiving DAAAs, and assuming treatment effectiveness of 95%, the HCV-RNA-positive prevalence could be considered to be 1.4%. Reasons for not receiving anti-HCV therapy in 18 patients included physician decision (N=5), loss to follow-up (N=5), patient refusal (N=2), and unknown reasons (N=4). Of the 29 HCV-RNA-positive patients, the infection was chronic in 24, acute/recent in 1, and unknown duration in 4. HCV-related liver cirrhosis was present in 71 (5.4%) PLWH overall, and 3 (10.3%) HCV-RNA-positives, and 68 (23.4%) of those who cleared HCV after anti-HCV therapy (P=.04). The prevalence of HCV Ab decreased steadily from 37.7% in 2015 to 28.6% in 2019 (P <.001). Likewise, HCV-RNA prevalence decreased from 22.1% in 2015 to 22.1% in 2019 (P <.001). Anti-HCV treatment uptake increased from 53.9% in 2015 to 95.0% in 2019 (P <.001) (Table).

Conclusion: Active HCV infection among PLWH in Spain at the end of 2019 was 2.2%, that is, 90.0% lower than in 2015, meaning that elimination of HCV infection among PLWH in Spain is an achievable goal shortly. Increased exposure to DAAs was likely the main reason for this sharp decrease. Despite the high coverage and effectiveness of DAA-based treatment, HCV-related cirrhosis among those successfully treated for HCV remains significant in this population.

Table. Trends in HIV/HCV coinfection in Spain, 2015-2019

<table>
<thead>
<tr>
<th>Year</th>
<th>Centers</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>2016</td>
<td>35,791</td>
<td>38,994</td>
</tr>
<tr>
<td>2017</td>
<td>1,067</td>
<td>1,088</td>
</tr>
<tr>
<td>2018</td>
<td>98.7%</td>
<td>99.0%</td>
</tr>
<tr>
<td>2019</td>
<td>37.7%</td>
<td>34.6%</td>
</tr>
<tr>
<td>HCV Ab-positive</td>
<td>22.1%</td>
<td>11.8%</td>
</tr>
<tr>
<td>HCV-RNA-positive</td>
<td>59.3%</td>
<td>74.7%</td>
</tr>
<tr>
<td>Anti-HCV treatment uptake</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
442 NO CHANGE IN INCIDENCE OF RECENTLY ACQUIRED HCV IN HIV+ MSM IN GERMANY (NOCo COHORT)

Patrick Ingiliz1, Natasha Martin2, Thomas Lutz3, Knud C. Scheve4, Stefan Mauss5, Stefan Christensen6, Sonia Jain7, Feng He8, Martin Daumer8, Axel J. Schmidt9, Michael Sabranski10, Axel Baumgarten11, Markus Bickel12, Jürgen K. Rockstroh13, Christoph Boeckele14

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Background: Direct-acting antiviral agents (DAA) against the hepatitis C virus (HCV) have been available in Germany since February 2014. Men who have sex with men (MSM) have been identified as one subgroup with continuous HCV transmission and as a target for HCV micro-elimination efforts. We assess newly acquired HCV among MSM in Germany.

Methods: The German NOCo cohort consists of patients from six German HIV and hepatitis C treatment sites providing care for more than 8000 HIV-positive MSM, and serving as primary care providers and HIV pre-exposure prophylaxis (PrEP) sites. Patients who were diagnosed with recently acquired HCV infection since 2014 were enrolled. Virologic data, HIV and HCV treatment data, risk factors and behavior as well as liver disease assessment is acquired regularly.

Results: Between January 2014 and September 2020, 214 MSM with recently acquired HCV infection were included. A majority were Caucasian (94%), and mean age was 45.3 years (standard deviation, SD, 9.6). At HCV diagnosis, median ALT level was 201 U/L (interquartile range, IQR, 86 – 509), and median HCV viral load was 483,028 IU/mL (IQR 77,804 – 2,525,000). The most prevalent HCV genotype was 1a (58.9%), followed by genotype 4d (16.4%), and 3a (6.1%). The risk factors for HCV acquisition were as follows: MSM: 92.5%, intravenous drug use: 2.8%, intranasal drug use: 0.9%, other: 0.5%. A subgroup of 17 (7.8%) MSM were not co-infected with HIV, of whom 10 (58.8%) were using PrEP. In 198/214 (92.5%) patients outcome data were available: DAA treatment was documented in 148 patients (74.7%), 16/198 (8.1%) had a spontaneous clearance, and in 34 patients (17.2%) treatment was not started, in most cases (35.3%) due to health insurance constraints. Among those treated, DAA was initiated a median 6.6 months (IQR 3.4 to 9.6) after diagnosis; all treated patients achieved a sustained virologic response (SVR), or treatment was still ongoing (14%). Between 2014-2019, 26-36 patients were diagnosed with recently acquired HCV annually. In relation to all HIV-positive MSM under care, the incidence was 0.32 – 0.39% per year with no significant change over time.

Conclusion: In this preliminary analysis from the German NOCo cohort, HCV incidence remained stable despite a broad use of DAs. Delays to HCV treatment initiation and health constraints may fuel ongoing HCV transmission, as well as continuous or even increasing risk behavior.

443 HEPATITIS C CASCADE OF CARE IN HIV/HCVC-OINFECTED PERSONS IN EUROPE IN THE DAA ERA

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Background: The WHO global hepatitis C (HCV) elimination targets include diagnosing 90% and treating 80% of HCV-infected individuals by 2030. We described the HCV cascade of care (CoC) for people living with HIV (PLHIV) across Europe to assess progress towards reaching these two targets.

Methods: HIV/HCV-coinfected participants in the EuroSIDA cohort under prospective follow-up at 1 October 2018 were described using a CoC with 3 diagnostic stages - anti-HCV positive, ever HCV RNA tested, currently HCV RNA positive; and 7 treatment stages - ever chronically infected (multiple imputation for persons with missing HCV RNA test data), ever diagnosed with chronic HCV, ever treated, completed treatment, sufficient follow-up available, follow-up HCV RNA available, cured. CoC were compared across five European regions and 20 countries enrolling >50 persons.

Results: Of 5,080 anti-HCV positive persons, 4,609 (90.7%, 95% confidence interval (CI) 89.9-91.6) were ever HCV RNA tested (Figure 1A) and 24.3% of individuals (95% CI 21.9-26.7) were currently HCV RNA positive, with higher prevalence in Central-East and East (45.4% and 33.2%, respectively). Among all participating countries the proportion of currently HCV RNA positive was the highest in Estonia (62% of 160 anti-HCV positive) and lowest in Austria (4.8% of 124). An estimated 4,562 (89.8%, 95% CI 88.9-90.7) anti-HCV-positive individuals have been ever chronically infected, of which 4,155 persons (90.1%, 95% CI 89.2-91.0) have been ever diagnosed with chronic HCV (Figure 1B). In Eastern Europe, 68.4% of chronic infections have ever been diagnosed, with >93% in the other four regions. Overall, 2,989 persons have ever been treated (65.5% of the ever chronically infected, 95% CI 63.8-67.2) and 2,186 individuals (47.9% of the ever chronically infected, 95% CI 45.8-50) were cured. Cure proportion ranged from 6.7% of 356 ever chronically infected in Belarus to 87.2% of 109 in Austria.

Conclusion: In all regions except Eastern Europe, >90% of anti-HCV positive PLHIV under follow-up at 1 October 2018 have been tested for HCV RNA. In South and Central-West, >80% ever chronically infected PLHIV have ever received treatment. Overall, the proportion with cured HCV infection did not exceed 80% in any region, and was significantly different between countries. Increased access to affordable direct acting antivirals, particularly in Eastern Europe, is required to achieve HCV elimination by 2030 among PLHIV in Europe.

444 ADEQUATE DACLATASVIR EXPOSURES IN CHILDREN 14-35 KG WITH AVAILABLE ADULT FORMULATIONS

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1Chiang Mai University, Chiang Mai, Thailand, 2Cairo University, Cairo, Egypt, 3PENTA Foundation, Padova, Italy, 4University of Florence, Florence, Italy, 5World Health Organization, Geneva, Switzerland, 6Ain Shams University, Cairo, Egypt

Background: World Health Organization 2016 guidelines recommend Sofosbuvir (SOF)/Daclatasvir (DCC) as a pangenotypic regimen for the treatment of adults with chronic HCV infection. SOF/DAC is widely available as low-cost generic formulation in low and middle-income countries (LMICs). Recent studies in adolescents (≥12 to <18) using SOF/DCC 400/60 mg once-daily (OD) adult dose reported excellent efficacy and safety. DCC pharmacokinetic (PK) data in younger children are lacking. Within the framework of the Global Accelerator
for Pediatric Formulations (GAPF), we performed a population PK analysis using data from adolescents to predict DCV exposure in children <35 kg to determine the lowest body weight children could be treated with the available DCV formulations (60 and 30 mg).

Methods: Data from HCV-infected adolescents receiving SOF/DCV (400/60 mg OD) who participated in a PK study in Egypt were used for PK model development. Intensive PK sampling was performed pre-dose, then 0.5, 1.0, 1.5, 2, 4, 8, 12, and 24 hrs post-dose. PK parameters were estimated using a population approach (NONMEM VII). Monte Carlo simulations were run for virtual children between 10 to <35 kg receiving 60 mg or 30 mg OD and DCV exposures (AUC0-24) were compared with the expected adults range (6.15 to 20.63 µg.hr/mL).

Results: Seventeen HCV-infected adolescents (13 males) provided 151 DCV concentrations. Median (range) age was 14 (11-18) years and weight 50 (32-63) kg. DCV plasma concentrations were best described by a 1-compartment model with transit absorption compartments. Body weight (allometrically scaled) and albumin influenced DCV PK parameters. DCV oral clearance and volume of distribution were 7.05 L/hr/70kg and 95.8 L/70kg. In adolescents using 60 mg DCV OD, mean (SD) DCV AUC0-24, Cmax and Clast were 12,004 (4,916) ng.hr/mL, 1,182 (333) ng/mL and 194 (168) ng/mL, respectively; while predicted to be 9,808 (3,949) ng.hr/mL, 1.039 (316) ng/mL and 148 (129) ng/mL in children 17 to <35 kg receiving 30 mg OD. Simulations showed that the proportion of children with DCV exposures above expected range rapidly increased for children <30 kg using 60 mg OD; and similarly for children 10-14 kg using 30 mg (Fig 1).

Conclusion: DCV 30 mg OD is expected to provide exposures comparable to adult values in children 14-35 kg. Our results suggest that children could be treated using currently available low-cost DCV formulations together with approved doses of pediatric SOF formulations, thus expanding access to HCV treatment.

Figure 1: Predicted daclatasvir exposure (AUC0-24) in children receiving DAC 30 vs 60 mg OD over the weight range 10 to <35 kg.

445 FAILURE TO ACHIEVE HCV MICROELIMINATION AMONG PLWH IN SPAIN
Alejandro Gonzalez-Serna1, Juan Macias2, Rosario Palacios2, Cristina Gómez-Ayerve2, Francisco Tellez2, Antonio Rivero-Izquier2, Marta Fernandez-Fuertes2, Jesús Santos2, Luis M Real1, Gonzalez-Domenech Carmen Maria1, Jesus Gomez-Mateos1, Juan A. Pineda1

1Hospital Universitario de Valme, Seville, Spain, 2Hospital Virgen de la Victoria, Malaga, Spain, 3Hospital de Puerto Real, Puerto Real, Spain, 4Hospital Universitario Reina Sofia, Cordoba, Spain

Background: Spain is close to HCV microelimination, so rates of recently acquired HCV infection (RAHC) should decrease. Nowadays, men who have sex with men (MSM) carry the highest risk of HCV acquisition. Our aim was to estimate the incidence of and the factors associated with RAHC, together with reinfection rates, among patients infected with HIV through sexual intercourse.

Methods: This was a prospective cohort study conducted at four hospitals in Spain. MSM and non-MSM infected with HIV through sexual intercourse patients consecutively attending these hospitals were included. Primary RAHC infection was diagnosed when anti-HCV antibody seroconversion was documented. In anti-HCV positive patients, initially without HCV viremia, a diagnosis of reinfection was established if plasma HCV RNA was detected.

Results: Three hundred and fifty patients tested negative for anti-HCV at baseline and had at least one follow-up visit. Among them, there were 16 RAHC cases from 2016 to 2019. RAHC incidence rates [IR (95% CI)] per 100 person-years (py) were 3.77 (0.5-12.9) in 2016, 1.85 (0.6-4.3) in 2017, 1.49 (0.4-3.8) in 2018 and 1.98 (0.6-4.5) in 2019 (Figure 1A). Only previous sexually transmitted infections (IRR: 18.23; 95% CI 1.93-172.1; p=0.011), male sex (IRR: 8.33; 95% CI 1.3-54; p=0.026) and sharing chem-sex drugs (IRR: 4.93; 95% CI 1.17-20.76; p=0.030), were independently associated with RAHC. Four of 42 (9.5%) patients became reinfected (Figure 1B).

Conclusion: The incidence of RAHC among HIV-infected patients showed a decrease after 2016, although a lower but steady incidence of residual cases still remains. HCV reinfections showed a similar pattern. New infections were associated with sharing chem-sex drugs among MSM. This suggests that the elimination of HCV infection in patients infected with HIV through sexual intercourse slowed down since 2017, likely due to sexual contacts with HCV-infected MSM without HIV-infection who remain undiagnosed.

Figure: 1

446 SELF-TESTING FOR HCV: MULTICOUNTRY EVIDENCE ON USABILITY AND ACCEPTABILITY
Elena Ivanova Reipold1, Thi Thuy Van Nguyen1, Gamal Shihaa1, Keteven Stvilia1, Aliza Monroe-Wise1, Cheng Wang2, Muhammad Jamil1, Cheryl C. Johnson3, Philippa Easterbrook4

1Foundation for Innovative New Diagnostics, Geneva, Switzerland, 2World Health Organization, Vietnam Country Office, Hanoi, Viet Nam, 3Manuscript University, Mansu, Egypt, 4National Center for Disease Control, Tbilisi, Georgia, 5University of Washington, Seattle, WA, USA, 6Guangxi Medical University, Nanning, China, 7World Health Organization, Geneva, Switzerland

Background: Globally, < 20% of all persons with hepatitis C (HCV) infection have been tested and only one quarter of diagnosed persons have been treated. Self-testing for HCV antibodies (HCVST) may be an additional strategy to expand access to HCV testing and support elimination efforts. We undertook studies to assess usability and acceptability of HCVST in general population as well as key populations: men who have sex with men (MSM) and people who inject drugs (PWID).

Methods: Observational studies were conducted in five countries: Egypt (general population); China (MSM); Kenya (PWID); Vietnam and Georgia (PWID and MSM). Oral fluid OraQuick® HCV Rapid Antibody Test with Instructions for Use (IFU) adapted for ST was used as a prototype HCVST kit. Participants were provided written and pictorial IFU and then conducted ST in a private room with a trained observer. In Egypt, in addition to IFU, study personnel provided a one-to-one demonstration on how to use the test. Usability was evaluated through observer assessment of errors and difficulties during ST using a standardized checklist; and acceptability using a semi-structured questionnaire. HCVST results were read and interpreted by participants and then re-read by the observer. All participants were re-tested with a professional use OraQuick® HCV Test performed by a trained provider.

Results: A total of 775 participants were enrolled across five studies. Participants completing all testing steps without any mistakes was greatest in Egypt and Georgia (>70%), and lowest in PWID from Kenya (30%) and Vietnam (46%). The most common error was incorrect sample collection. Inter-reader agreement ranged from 86% to 99%, and inter-operator agreement from 85% to 99%. The majority of PWID from Vietnam and Kenya required assistance in performing HCVST. The proportion of participants who found the kit very easy or acceptable using a semi-structured questionnaire. HCVST results were read and interpreted by participants and then re-read by the observer. All participants were re-tested with a professional use OraQuick® HCV Test performed by a trained provider.

Figure: 1

These are the first studies globally to demonstrate high usability and acceptability of HCVST in general population as well as key populations: men who have sex with men (MSM) and people who inject drugs (PWID).
consideration as it may be a promising tool for increasing coverage and achieving elimination goals.

<table>
<thead>
<tr>
<th>Egypt</th>
<th>China</th>
<th>Vietnam</th>
<th>Georgia</th>
<th>Kenya</th>
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<td>Self-reporting</td>
<td>MSM</td>
<td>PWD</td>
<td>MSM</td>
<td>PWD</td>
</tr>
<tr>
<td>Components of testing correctly without errors</td>
<td>102 (98%)</td>
<td>55 (55%)</td>
<td>48 (48%)</td>
<td>71 (69%)</td>
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<tr>
<td>Components of testing correctly without fillies</td>
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<td>43 (41%)</td>
<td>30 (29%)</td>
<td>50 (54%)</td>
</tr>
<tr>
<td>Assistance provided</td>
<td>12 (11%)</td>
<td>4 (4%)</td>
<td>76 (7%)</td>
<td>13 (12%)</td>
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<td>Informed consent agreement</td>
<td>80%</td>
<td>97%</td>
<td>95%</td>
<td>99%</td>
</tr>
<tr>
<td>Informed consent agreement</td>
<td>9.6%</td>
<td>98%</td>
<td>58%</td>
<td>100%</td>
</tr>
<tr>
<td>PARTICIPANT FEEDBACK</td>
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<td>n=100</td>
<td>n=105</td>
<td>n=101</td>
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<td>Total self-testing procedure any/any way</td>
<td>57 (49%)</td>
<td>64 (64%)</td>
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<td>94 (94%)</td>
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<td>Concerned about HCV transmission</td>
<td>62 (52%)</td>
<td>54 (54%)</td>
<td>102 (97%)</td>
<td>103 (100%)</td>
</tr>
<tr>
<td>Would use the self-test again</td>
<td>105 (89%)</td>
<td>76 (76%)</td>
<td>90 (90%)</td>
<td>92 (92%)</td>
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<tr>
<td>Would take the self-test to finally test</td>
<td>103 (88%)</td>
<td>70 (71%)</td>
<td>104 (100%)</td>
<td>105 (100%)</td>
</tr>
</tbody>
</table>

447 DRUG-USE STIGMA AND HEPATITIS C VIRUS INFECTION AMONG PWID IN INDIA

Eshan U. Patel1, Sunil S. Solomon1, Gregory M. Lucas1, Allison M. McFall1, Aylur K. Sirkrishnan2, Muniratnam S. Kumar1, Oliver Laeyendecker3, David C. Celentano4, David L. Thomas4, Thomas Quinn3, Shrutl H. Mehta1

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Background: Although drug use stigma is globally pervasive, empirical evidence of its role in hepatitis C virus (HCV) transmission is limited. We measured the association between drug use stigma and active HCV infection among community-based people who inject drugs (PWID) in India.

Methods: Between 8/2016 and 5/2017, a cross-sectional sample of PWID was recruited from 12 Indian cities (~1000/city) using respondent-driven sampling. Participants were ≥18 years old and reported injection drug use (IDU) in the past 2 years. Four domains of drug use stigma were examined (internalized, felt/normative, vicarious, and enacted) with 5-6 items/domain. Each subscale had a range of 0-3 with higher scores reflecting greater stigma (Cronbach’s α: 0.85-0.92). For each subscale, multivariable logistic regression with a random-intercept for each city was used to estimate adjusted odds ratios (aOR) of active HCV infection (aOR=1.18 [95%CI=1.04-1.33]), as did PWID with internalized stigma (aOR=1.52 [95%CI=1.26-1.83]), independent of age, gender, education, homelessness, incarceration, and aOR=1.52 [95%CI=1.26-1.83], respectively), independent of age, gender, and aOR=1.52 [95%CI=1.26-1.83], respectively), independent of age, gender, education, homelessness, incarceration, alcohol dependence, and HIV status. PWID reporting any enacted stigma had significantly greater odds of active HCV infection (aOR=1.18 [95%CI=1.04-1.33]), as did PWID with internalized stigma scores in the highest quartile (vs. lowest quartile; aOR=1.62 [95%CI=1.09-2.40]), independent of age, gender, education, homelessness, incarceration, alcohol dependence, frequency of IDU, ever sharing needles, ever participating in medication for opioid use disorder or syringe service programs, and HIV status.

Conclusion: PWID reporting enacted and internalized drug use stigma were significantly more likely to be living with HCV infection, suggesting stigma may play a role in HCV transmission and impede efforts to achieve HCV elimination. Strategies to reduce drug use-related stigma and discrimination are warranted.

448 ACCESS TO HCV CARE AMONG HIV/HCV-COINFECTED PEOPLE WHO INJECT DRUGS ACROSS CANADA

Charlotte Lanèe Delaunay1, Mathieu Maheu-Giroux1, Gayatri Marathe1, Sahar Saeed2, Curtis Cooper3, Sharon Walmsley4, Joseph Cox5, Mark Hull5, Valérie Martel-Laferrière5, Marina B. Klein5

1McGill University, Montreal, Canada, 2Washington University in St Louis, St Louis, MO, USA, 3University of Ottawa, Ottawa, Canada, 4University of Toronto, Toronto, Canada, 5Research Institute of McGill University Health Centre, Montreal, Canada, 6University of British Columbia, Vancouver, Canada, 7Centre de Recherche du CHUM, Montreal, Canada

Background: In North America, 81% of new HCV infections occur among people who inject drugs (PWID), and coinfection with HIV can exacerbate disease severity. Quantifying unmet needs in HCV prevention and treatment among HIV-HCV coinfected PWID is key for developing appropriate interventions to eliminate HCV as a public health threat by 2030. We investigated temporal trends in 1) HCV treatment uptake and efficacy; 2) injection practices; and 3) access to harm reduction programs among HIV-HCV coinfected PWID in Canada from 2003 to 2019.

Methods: We used data from the Canadian Coinfection Cohort, a prospective cohort study of 2,004 HIV-HCV coinfected patients. We included 1,090 participants from Quebec, Ontario, Saskatchewan, and British Columbia who reported injecting at least once between 2003 and 2019. Trends were examined using 3 time periods based on HCV treatment guidelines: 2003-2010: interferon/ribavirin-based; 2011-2013: 1st generation direct-acting antivirals (DAAs); and 2014-2019: 2nd generation DAAs. The harm reduction services assessed include needle and syringe programs (NSP), opioid agonist therapy (OAT), and supervised injection sites (SIS).

Results: Of 11,663 participants (median age: 30 years; 94.2% male; 33% were Indigenous. The overall HCV treatment rate among HCV RNA-positive people increased from 7 per 100 person-years (95%CI: 5-9) in 2003-2010 to 20 per 100 PY (95%CI: 18-22) in 2014-2019. In the 2nd generation DAA era, treatment efficacy was >90%, compared to 57% in 2003-2010. Cocaine remained the most frequently injected drug among active PWID, but its consumption decreased from 84% (95%CI: 83-86) of visits in 2003-2010 to 57% (95%CI: 56-59) in 2014-2019. Opioid injection increased from 50% (95%CI: 47-52) of visits in 2003-2010 to 60% (95%CI: 58-61) in 2014-2019. Report of needle/syringe sharing declined from 12% (95%CI: 11-15) in 2003-2010 to 6% (95%CI: 4-8) in 2014-2019, yet paradoxically, report of NSP also decreased (Figure). This might reflect a reduction in number of daily injections due to reduced cocaine use. OAT engagement among opioid injectors was also low (<20%), with no clear temporal trend. SIS data became available in 2014-2019 (reported at 9% of visits (95%CI: 8-10)).

Conclusion: HCV treatment access and outcomes have greatly improved among coinfelected PWID. Yet, exposure to injection-related risks continues and is increasingly related to opioid use. There is a need to maximize access to proven harm reduction strategies to prevent HCV re-infection and overdose.
449  FREQUENT HBsAg CLEARANCE DURING TENOFIVIR THERAPY IN HIV/HBV COINFECTION

Charles Béguelin1, Bernard Surial1, Eveline Hofmann1, Matthias Cavassini1, Huldrych F. Günthard1, Manuel Battegay3, Enos Bernacconi1, Patrick Schmid1, Alexandra L. Calmy1, Franziska Suter-Riniker2, Andri Rauch1, Gilles Wandelé1, for the Swiss HIV Cohort Study

1University Hospital of Bern, Bern, Switzerland, 2Lausanne University Hospital, Lausanne, Switzerland, 3University Hospital Zurich, Zurich, Switzerland

Background: Among persons with hepatitis B virus (HBV)-monoinfection, loss of the hepatitis B surface antigen (HBsAg), also described as HBV functional cure, is a rare event but associated with reduced incidence of liver-related complications. We aimed to assess the proportion of HBV functional cure among persons with HIV/HBV-coinfection during long-term tenofovir-therapy who experienced HBV functional cure, and to evaluate the association between quantitative HBsAg (qHBsAg) levels and this outcome.

Methods: All Swiss HIV Cohort Study participants with two positive HBsAg tests more than 6 months apart, and at least 4 years on tenofovir-containing antiretroviral therapy (ART) were considered. Our main outcomes were the loss of HBsAg during the first 2 years of tenofovir therapy and until the latest available follow-up. We explored the association between qHBsAg levels at tenofovir start and HBsAg loss using multivariable logistic regression adjusted for gender, age, ethnicity, HIV transmission group, CD4 count (<350/μl), as well as for HBV suppression (<20 IU/ml) and low qHBsAg (<1000 IU/ml) at tenofovir start.

Results: A total of 272 patients were included. Median age at 41 years (IQR 36-46) and 221 (81%) were men. At tenofovir start, 110 (49%) patients were hepatitis B envelope antigen (HBsAg) positive, 229 (84%) had detectable HBV-DNA (median 1050 IU/ml, IQR 89-1,1x10^6) and 217 (75%) had low qHBsAg. HBsAg loss was observed in 8% (19/230) of participants during the first 2 years of tenofovir-containing ART and in 16% (43/262) of them after a median follow-up time of 8.4 years (IQR 2.6-15.8). At the late follow up, 54% (16/27) of those with HBsAg loss seroconverted for Anti-HBs antibodies. In multivariable analysis, low qHBsAg at tenofovir start (OR 12.01, CI 2.50-57.71) as well as female gender (OR 9.15, CI 1.08-77.45) were significant predictors of HBsAg loss, whereas this outcome was less likely among participants with negative baseline HBV DNA (OR 0.14, CI 0.02-0.79).

Conclusion: We found high rates of HBsAg loss in PLWH coinfected with HBV on tenofovir-containing ART, and baseline quantitative HBsAg level was a strong predictor of this outcome.

450  HBV REPLICATION DURING TENOFIVIR THERAPY IS FREQUENT IN HIV/HBV COINFECTION

Eveline Hofmann1, Bernard Surial1, Matthias Cavassini1, Huldrych F. Günthard1, Manuel Battegay3, Enos Bernacconi1, Patrick Schmid1, Alexandra L. Calmy1, Franziska Suter-Riniker2, Andri Rauch1, Gilles Wandelé1, for the Swiss HIV Cohort Study

1University Hospital of Bern, Bern, Switzerland, 2Lausanne University Hospital, Lausanne, Switzerland, 3University Hospital Zurich, Zurich, Switzerland

Background: In persons living with HIV (PLWH) coinfected with hepatitis B virus (HBV), a tenofovir (TDF) containing antiretroviral therapy (ART) is the treatment of choice. Despite this treatment, certain patients exhibit incomplete HBV suppression. Ongoing viral replication has a negative impact on achieving functional cure, accelerates fibrosis, and is associated with a higher risk of developing hepatocellular carcinoma. In this study, we aimed to describe the proportion of PLWH with persistent HBV replication despite tenofovir and to identify associated risk factors.

Methods: We included all PLWH coinfected with HBV from the Swiss HIV Cohort Study, defined as two positive hepatitis B surface antigen (HBsAg) tests more than 6 month apart, with at least four years of TDF therapy and a positive HBsAg at TDF start. Patients who had an HIV RNA >200 cp/ml at two years were considered to have suboptimal adherence to ART and were therefore excluded from the analyses. We determined the proportion of patients with persistent hepatitis B viremia (HBV DNA >20 IU/ml) after two years and at the latest follow-up, and explored related risk factors using multivariable logistic regression adjusted for gender, age, ethnicity, CDC Stage 3, CD4 count at TDF start, HBV DNA levels at TDF start, previous HBV treatment, and hepatitis D coinfection.

Results: After the exclusion of 21 individuals with replicating HIV and 29 with missing HIV RNA measurements at two years, 222 PLWH were included in our analyses. Median age was 41 years (IQR 36-47), 179 (81%) were men, and median follow-up was 8 years (IQR 5.2-11.0). At TDF start, 187 (84%) had detectable HBV DNA, and 129 (58%) had high-level viremia (HBV DNA >2000 IU/ml). Persistent hepatitis B viremia was present in 61/222 (27%) at two years, and in 39/222 (18%) at the latest follow-up. At two years, 6/61 (10%) patients with persistent hepatitis B viremia had high-level viremia, and S/39 (13%) at the latest follow-up. In multivariable analysis, persistent hepatitis B viremia at two years was associated with CDC Stage 3 (OR 2.80, CI 1.18-6.64), and high HBV DNA levels at TDF start (OR 1.23, CI 1.07-1.44), whereas it was less likely in individuals with age over 40 years (OR 0.42, CI 0.18-0.97), or with hepatitis D coinfection (OR 0.06, CI 0.01-0.66, Figure).

Conclusion: In this nationwide cohort of PLWH coinfected with HBV, persistent hepatitis B replication after two years of TDF was frequent and associated with HBV DNA levels at TDF start and hepatitis D status.
Hepatitis Delta Infections Among Persons With HIV in Europe

A high prevalence of hepatitis delta virus (HDV) infection, the most severe form of viral hepatitis, has been reported among persons with HIV (PHIV) and hepatitis B virus (HBV) infection in European cohorts. We analyzed data from two large HIV cohorts to characterize the current epidemiological trends in HDV infections across Europe.

Methods: All PHIV with a positive hepatitis B surface antigen (HBsAg) test in the Swiss HIV cohort Study and EuroSIDA were considered and tested for anti-HDV antibodies. HBV DNA amplification was performed in anti-HDV-positive patients. Demographic and clinical characteristics at initiation of antiretroviral therapy were compared between HDV-positive and HDV-negative individuals using descriptive statistics. The associations between HDV infection and overall mortality, liver-related mortality as well as hepatocellular carcinoma (HCC) were assessed using Kaplan-Meier and multivariable Cox regression adjusted for age, gender, HIV transmission group, baseline CD4 and cohort.

Results: Of 2793 HBsAg-positive patients, 1556 (56%) had stored serum available and were included. The prevalence of HDV co-infection was 15.2% (237/1556; 95% CI: 13.5-17.1%), of whom 66% (132/200) had active HDV replication. Anti-HDV antibody positive prevalence ranged from 32.8% (95% CI: 24.7%-41.7%) in Eastern Europe, to 29.7% (95% CI: 21.4%-39.1%) in the South and 14.9% (95% CI: 12.7%-17.6%) in Northwestern Europe. HDV-positive persons were more likely to be persons who inject drugs (PWID) (76.8% vs. 14.3%, p<0.001) and to have positive hepatitis C serumore (75.5% vs. 24.3%, p<0.001), compared to those without HDV-infection. Among PWID, the prevalence of HDV co-infection was 49.2%, with similar estimates across the three regions. During a median follow-up time of 9.8 years [IGR 4-16.6], seventy-five (31.6%) HDV-positive patients and 261 (19.8%) HDV-negative individuals died. 43% (32/75) of the deaths were liver related in HDV-positive patients compared to 18% (46/261) in HDV-negative individuals. HDV infection was associated with overall mortality (adjusted hazard ratio 1.4; 95% CI 1.1-1.95, p=0.03), liver-related death (2.9, 1.6-5.1, p<0.001) and hepatocellular carcinoma (6.5, 2.6-16.6, p<0.001).

Conclusion: Hepatitis delta prevalence among PWI in Europe varies strongly across regions and is particularly high in PWIDs. This could reflect different availability of harm reduction programs. HDV coinfection is associated with increased mortality and liver related events including HCC.

Table 1: Demographic and clinical characteristics of the cohort

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PHIV N=195</th>
<th>NPHIV N=105</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Age - median (IQR)</td>
<td>28 (21-43)</td>
<td>27 (23-37)</td>
<td>0.03</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>36 (41)</td>
<td>52 (50)</td>
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</tr>
<tr>
<td>Female</td>
<td>64 (59)</td>
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<tr>
<td>Black</td>
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<tr>
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<td>7 (7)</td>
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<td>Non-Hispanic</td>
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<tr>
<td>Duration of HIV Infection (Years)</td>
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<td>3.5 (2.8 - 10.3)</td>
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<td>Other immunosuppressive Condition</td>
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<tr>
<td>Yes</td>
<td>3 (3)</td>
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<tr>
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<td>92 (97)</td>
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<td>61 (64)</td>
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</tbody>
</table>

PHIV: perinatally-acquired HIV; NPHIV: non-perinatally-acquired HIV; copies per milliliter; HBV: hepatitis B virus; iAB: hepatitis B surface antibody

453 RPV ACTIVATES STAT1 IN STELLATE CELLS TO REGULATE LIVER INJURY IN PLHIV AND NAFLD

Maria Luisa Montes1, Carmen Busca Arenzana1, Angela B. Moragrega, Nadezda Apostolova2, Antonio Oliva3, Luz Martinez Carbonero1, Eugenia Moreno1, Jose I. Bernardino1, Ignacio Perez-Valero1, Juan Gonzalez-Garcia1, Juan V. Estigues1, Jose R. Arribas2, Ana Blas-Garcia1

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Background: A high prevalence of chronic liver disease has recently shown Rilpivirine (RPV) selectively triggers hepatic stellate cell (HSC) inactivation and apoptosis through signal transducer and activator of transcription (STAT)-1-mediated pathways. These actions clearly attenuate liver inflammation and fibrosis, regardless of the etiology of liver injury. We studied the effects of this RNIRI on hepatic steatosis, inflammation and fibrosis in liver biopsies of HIV-infected subjects with identified NAFLD.

Methods: In a cross-sectional study, we analyzed 42 well-controlled PLHIV subjects diagnosed with NAFLD by liver biopsy. Histological analysis was performed in 3μm paraffin-embedded liver sections by H&E staining and immunohistochemistry. Nuclear expression of STAT-1 was quantified and compared between subjects with and without RPV-based therapy. A log transformation (Ln) of the STAT-1 values was applied. Differences in LnSTAT-1 were assessed using factorial analysis of variance, considering exposure to RPV and diagnoses of steatosis, steatohepatitis and fibrosis as inter-subject factors, and exposure time to RPV and BMI as covariates.

Results: Studied subjects were 100% male, median age 49 (44-54) years, median CD4 count 802 (608-940) cells/µL, and 60% of them had metabolic syndrome. All subjects were receiving ART with undetectable HIV viral load and 45% were receiving RPV-based therapy. Liver biopsies showed 43% steatosis >30%, 60% steatohepatitis and 43% fibrosis >1. Detection of nuclear STAT-1 expression in non-parenchymal cells revealed a significant association of RPV-based therapy with enhanced activation of this transcription factor in hepatic sections of patients with identified liver injury. The protective effect of promoting STAT1-dependent HSC inactivation was observed in patients with different stages of NAFLD, from mild/intense steatosis to steatohepatitis or fibrosis. Interestingly, the increase in STAT1 activation observed in those exposed to RPV-based therapy was also evident in F0 subjects, probably due to the presence of steatosis or steatohepatitis among these subjects.

Conclusion: PLHIV with NAFLD who receive RPV-based therapy showed increased STAT1 activation, pointing to ongoing HSC inactivation and apoptosis to reduce the progression of hepatic damage. Our results suggest RPV-based ART would be especially indicated in HIV-infected patients with NAFLD-derived liver injury to prevent liver fibrosis and inflammation.
454 RELATIONSHIP BETWEEN ALCOHOL USE AND SUSTAINED VIROLOGIC RESPONSE TO HCV DAA THERAPY

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¹Atlanta VA Medical Center, Decatur, GA, USA, ²London School of Hygiene & Tropical Medicine, London, UK, ³Yale University, New Haven, CT, USA, ⁴University of Pennsylvania, Philadelphia, PA, USA

Background: Some payors include alcohol abstinence as a requirement for reimbursement of direct-acting antiviral (DAA) therapy for chronic hepatitis C virus (HCV) infection. However, it remains unclear if alcohol use is associated with lower likelihood of sustained virologic response (SVR) to DAA therapy. We examined the relationship between alcohol use, assessed within the 18 months prior to DAA therapy, and SVR.

Methods: Using the Veterans Health Administration (VA) Birth Cohort, which includes all Veterans born between 1945 and 1965, we identified HCV patients dispensed DAA therapy between 1 January 2014 and 31 December 2018. Alcohol use (abstinent, abstinent with history of alcohol use disorder [AUD], lower risk, moderate risk, high risk) was determined using a combination of self-reported score from the Alcohol Use Disorders Identification Test—Consumption instrument and validated International Classification of Diseases, 9th/10th Revision AUD diagnoses. We assessed SVR, defined as undetectable HCV RNA ≥12 weeks after DAA therapy, through May 31, 2019. Multivariable logistic regression was used to determine odds ratios (ORs) with 95% confidence intervals (CIs) of SVR for each alcohol use category compared to lower risk, adjusting for HCV genotype, HBV coinfection, and hepatic decompensation. A stratified analysis was performed by HIV status.

Results: Among 77,046 HCV patients dispensed DAA therapy (mean age, 63 years; 97% male; 50% white race; 83% HCV genotype 1; 3% HIV positive), alcohol use prior to DAA therapy varied: abstinent (47%), abstinent with AUD (13%) lower risk (19%), moderate risk (5%), and high risk (17%). Overall, 94% of patients achieved SVR. We observed no association with SVR among patients whose alcohol use was classified as abstinent (OR 1.06, 95% CI 0.96-1.16), abstinent with history of AUD (OR 0.91, 95% CI 0.81-1.03), moderate risk (OR 0.97, 95% CI 0.89-1.12), or high risk (OR 1.00, 95% CI 0.89-1.12) relative to lower risk, adjusting for important demographic and clinical characteristics. Results were similar when stratified by HIV status; however, the HIV co-infected subset (n=2,372) was underpowered.

Conclusion: Level of alcohol use, even at the highest category of use, was not associated with a lower likelihood of achieving SVR after adjusting for important differences between the groups. These findings suggest that DAA therapy should be provided and reimbursed despite alcohol use. Restricting access to DAA therapy based on alcohol use is an unnecessary barrier to DAA therapy and challenges HCV elimination goals.

455 APPLICATION OF THE NOVEL FIBROSCAN-AST SCORE IN AN HIV-MON INFECTED COHORT

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Background: Non-alcoholic fatty liver disease (NAFLD) is becoming a serious complication for people living with HIV (PLWH). With validation of transient elastography (TE) and controlled attenuation parameter (CAP) as non-invasive tools in contrast to biopsies, diagnosing liver fibrosis and hepatic steatosis (HS) had become much easier. Quite recently the FibroScan-AST (FAST) score had been validated in HIV negative individuals with suspected NAFLD as an efficient tool to identify those at risk of progression to NASH. To date the FAST score had not been reported in HIV mono-infected individuals (HIV+).

Methods: We enrolled 432 HIV positive patients presenting at our outpatient clinic between August 2013 to December 2018. Liver stiffness and HS were assessed yearly by TE using an M-probe of FibroScan (Echosens, Paris, France). FAST Score was calculated retrospectively according to Newsome et al. for each patient and compared to other markers of liver disease.

Results: Overall, 303 HIV+ patients (79% male, average age at baseline 45.9±11.5 years, average duration of infection 9.3±6.4 years) with at least two visits were analysed. All patients were administered ART. FAST score was available for 187, 236, 222, 192 and 111 patients at visit V1, V2, V3, V4 and V5, respectively. Distribution of FAST Score and CAP values are shown in Fig. 1. FAST score at baseline showed a strong correlation with baseline FIB4 scores (R: 0.740; p<0.001) and APRI scores (R: 0,514; p<0.001) but was weaker with CAP values (R: 0.345; p<0.001). In contrast to an significant correlation with FIB4, APRI and CAP values. In contrast to an significant correlation with FIB4, APRI and CAP values. In contrast to an significant correlation with FIB4, APRI and CAP values.

Conclusion: This is the first report on FAST Score in HIV+ persons. We found a significant correlation with FIB4, APRI and CAP values. In contrast to an observed steady redistribution towards higher CAP values (reflecting an increase in steatosis) over five years, the FAST score remained unchanged in its distribution until there was a sudden increase towards a so far not well defined “grey area” of progressive NAFLD after four years of observation. Possible risk factors in univariable analyses for higher FAST scores were male sex, overweight, DM2 and certain antiretroviral drugs. Due to the rapid, inexpensive and non-invasive assessment of the FAST score, it should be further investigated in PLWH.
456 ARE MODERN ANTIRETROVIRALS HEPATOTOXIC? SIGNALS IN PATIENTS STARTING ART IN NA-ACCORD

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Background: Despite effective antiretroviral therapy (ART), rates of end-stage liver disease (ESLD) remain high. Specific ART drugs may add to the risk of ESLD but past analyses have studied prevalent users exposed to old hepatotoxic drugs and used inadequate methods for detecting risk signals in complex data. We sought to detect which components of modern ART might contribute to ESLD risk.

Methods: We selected patients from 12 cohorts contributing validated ESLD events to the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD). We followed these initiating ART after 1 Jan 2004 with a nucleos(t)ide backbone of either abacavir/lamivudine or tenofovir/emtricitabine and a modern third (anchor) drug until a first ESLD event, death, or end of a cohort’s ESLD validation period, loss to follow up or 31 Dec 2015. We estimated associations between cumulative exposure to each drug (per five years) and ESLD using a Bayesian Cox model akin to the FDA’s method for detecting associations between cumulative exposure to each antiretroviral drug component (per five years).

Conclusion: We show how new user cohort designs can be used to detect toxicity signals even with relatively few events. While modern ART poses less risk for ESLD than hepatitis coinfection, some drugs showed toxicity signals. Confirming whether these drugs contribute to ESLD risk requires designs that focus on causality.

Table

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Component</th>
<th>Prior HR</th>
<th>95% CI</th>
<th>Median</th>
<th>Prior HR</th>
<th>95% CI</th>
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<td>Boosted protease inhibitors</td>
<td>Atazanavir</td>
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<td>0.49 to 2.5</td>
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<td>Nevirapine</td>
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<td>0.32 to 7.1</td>
<td>1.5</td>
<td>0.51 to 4.2</td>
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<td>Nucleos(t)ide backbones</td>
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<td>Tenofovir</td>
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<td>0.25 to 4.0</td>
<td>0.78</td>
<td>0.36 to 1.8</td>
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457 IMPACT OF BINGE DRINKING ON MORTALITY AND LIVER DISEASE IN THE SWISS HIV COHORT STUDY

Bernard Surial1, Nicolas Bertholet2, Jean-Bernard Darpepp3, Katharine E. Darling4, Alexandra Calmy5, Huldrych F. Günthard2, Marcel Stöckle6, Enos Bernasconi7, Patrick Schmid8, André Rauch9, Hansjakob Furrer10, Gilles Wandeler11, for the Swiss HIV Cohort Study

1University Hospital of Bern, Bern, Switzerland, 2Lausanne University Hospital, Lausanne, Switzerland, 3University Hospitals of Geneva, Geneva, Switzerland, 4University Hospital Zurich, Zurich, Switzerland, 5University Hospital Basel, Basel, Switzerland, 6Servizio di Malattie Infettive, Lugano, Switzerland, 7St Gallen Cantonal Hospital, St Gallen, Switzerland

Background: Studies in the general population suggest that binge drinking is associated with an increased risk of death independently of the average daily consumption of alcohol. Considering the high prevalence of alcohol use disorder among persons with HIV, we assessed the impact of specific drinking patterns on mortality and the occurrence of liver events in the Swiss HIV Cohort Study.

Methods: We included all cohort participants with follow-up between January 2013 and April 2020. Based on responses to the routinely recorded Alcohol Use Disorder Identification Test (AUDIT-C), their drinking behavior was grouped into “abstinence” (no alcohol consumption), “non-hazardous drinking”, “hazardous but not binge drinking (AUDIT-C ≥3 in women and ≥4 in men)”, and “binge drinking” (≥5 drinks/occassion more than monthly). We estimated adjusted incidence rate ratios (IRR) of all-cause mortality, liver-related mortality and liver events (including the development of cirrhosis) for time-varying drinking patterns using multivariable quasi-Poisson regression, adjusted for demographic characteristics, comorbidities, smoking and illicit drug use.

Results: We included 11'849 individuals: 3'264 (27.5%) were female, median age was 46 years (IQR 38–53), 9'997 (86.8%) were Caucasian, 5'425 (45.8%) were men who have sex with men, 573 (4.8%) had hepatitis B coinfection and 1'116 (9.4%) hepatitis C coinfection. Over a median follow-up of 6.8 years (IQR 4.1–7.0), 470 individuals died (incidence rate [IR] 7.1/1'000 person-years [PY], 95% CI 6.5–7.8), of which 37 were liver-related (IR 0.6/1'000 PY, 95% CI 0.4–0.8), and 239 liver events occurred (IR 3.7/1'000 PY, 95% CI 3.2–4.2). Compared to non-hazardous drinking, binge drinking was associated with a higher rate of all-cause mortality (adjusted IRR 1.9, 95% CI 1.3–2.7) and liver events (adjusted IRR 3.8, 95% CI 2.4–5.8). All-cause (adjusted IRR 1.9, 95% CI 1.5–2.3) and liver-related mortality (adjusted IRR 3.9, 95% CI 1.7–9.3) were increased in individuals reporting abstinence compared to non-hazardous drinking. We observed no significant differences in outcome rates between non-hazardous and hazardous without binge drinking (Figure). Findings were consistent after excluding individuals with viral hepatitis.
458 HIV/HCV CONIFERTION INDUCES PRO-INFLAMMATARY PLASMA GLYCOMIC SIGNATURES

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Background: Glycans on circulating (plasma) glycoproteins and antibodies (IgGs) play an important role in regulating inflammatory responses. In particular, reductions in IgG galactosylation (agalactosylation; loss of the monosaccharide galactose) and sialylation (hyposialylation; loss of sialic acid) as well as induction of bisected GlcNAc glycans increase the pro-inflammatory function of IgGs, by enhancing FcγR binding. These glycomic features have been linked to the development and maintenance of several inflammatory diseases. However, their levels during HIV/HCV co-infection remain unclear.

Methods: We enrolled 249 HCV-exposed (HCV antibody positive) adults mean age 56±6 years, 36% female, with and without active HIV and/or active HCV infection in four groups: (52 HCV-/HIV-; 26 HCV-/HIV+; 113 HCV+/HIV-; and 58 HCV+/HIV+), from the AIDS Linked to the IntraVenous Experience (ALIVE) cohort. N-glycans from plasma and isolated IgG were analyzed using capillary electrophoresis. Kruskal-Wallis test was used for statistical analysis.

Results: HCV mono-infection was associated with inductions in levels of bisected GlcNAc glycans in plasma glycoproteins (FDR<0.002) compared to the HCV-/HIV- group (Figure A-C). Furthermore, HCV infection was associated with higher levels of the pro-inflammatory bisected GlcNAc glycans on IgG glycome (FDR<0.0003), compared to the HCV-/HIV- group (Figure D). HIV/HCV co-infection further increased these pro-inflammatory glycomic traits on plasma glycoproteins and IgGs when compared to mono-infection and the HCV-/ HIV- group (FDR<0.0002) (Figure D).

Conclusion: We identified novel host glycemic factors that may contribute to higher inflammation and immune activation during HCV/HIV co-infection. The potential prognostic and functional significance of these glycomic signatures in modulating the disease course during HCV/HIV co-infection warrants further investigation.

459 MITOCHONDRIAL DNA HAPLOGROUPS AND SPONTANEOUS HCV CLEARANCE: A MULTI-COHORT ANALYSIS

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Background: Hepatitis C virus (HCV) infects >70 million persons globally. Most persons with HCV have chronic infection and an increased risk of cirrhosis and its complications; a minority spontaneously clear infection. Polymorphisms in interferon lambda (including rs368234815, ∆G/TT) and other genes are estimated to explain ~15% of the variation in HCV spontaneous clearance (SC). Because mitochondria can regulate activation, differentiation, and survival of immune cells, we asked whether mitochondrial DNA (mtDNA) haplogroups were associated with SC in persons with and without HIV co-infection.

Methods: Data were from the HCV Genetics Consortium, a multi-cohort consortium including individuals of European (EA) and African (AA) genetic ancestry from 15 cohorts across North America and Europe. SC was defined as the presence of HCV antibody without HCV RNA in plasma. Genotyping was performed using the Illumina Omni1-Quad array and 24 mtDNA variants and HaploGrep were used to define haplogroups. In each ancestry group, we performed logistic regression analyses of mtDNA haplogroups and SC, both with stratification by HIV status and including HIV status as a covariate in separate models. Regression models also adjusted for rs368234815, (GT/TT) and other genes are estimated to explain ~15% of the variation in HCV spontaneous clearance (SC). Because mitochondria can regulate activation, differentiation, and survival of immune cells, we asked whether mitochondrial DNA (mtDNA) haplogroups were associated with SC in persons with and without HIV co-infection.

Results: The total sample included 2028 persons (1581 EA; 447 AA) with HCV. Within the ancestry groups, 68% and 48% were male, 16% and 50% had HIV co-infection, and 37% and 38% had SC, respectively. Overall haplogroup distribution was as expected for EA and AA populations. No statistically significant associations between haplogroups and SC among persons with HCV mono-infection were found. Among EA persons with HCV/HIV co-infection, mtDNA haplogroup I was more frequent in SC independent of rs368234815 and sex (6.8% vs.1.9%; adjusted OR 4.3; 95% CI 1.1-21.3, p=0.04). In models
including all EA persons with HCV and adjusting for HIV co-infection and sex, haplogroup I remained associated with SC (3.9% vs. 2.0%, adjusted OR 2.9; 95% CI:1.1-3.7, p=0.03).

**Conclusion:** In EA persons with HIV/HCV co-infection, a rare mtDNA haplogroup (~3%) was associated with HCV SC. Limitations included small sample sizes for non-EA and HIV co-infection analyses, limited mtDNA variants, and few covariates. While this finding cannot explain substantial SC variation at a population level, it does suggest mitochondrial mechanisms of SC that may be influenced by HIV co-infection. This association should be validated in other cohorts, and mechanisms explored in translational studies.

460 A SERIAL COMBINATION OF STEATOSIS NONINVASIVE MARKERS IN HIV-MONONEFECTED SUBJECTS

**Carmen Busca**, Matilde Sanchez-Conde, Marta Rosas, Eulalia Valencia, Ana Maria Moreno-Zamora, Virginia Moreno, Luz Martin-Carbonero, Santiago Moreno, Ignacio Perez-Valero, Jose I. Bernardino, Jose R. Arribas, Juan Gonzalez-Garcia, Antonio Olivera, Maria Luisa Montes

1Hospital La Paz Institute for Health Research, Madrid, Spain, 2Hospital Ramón y Cajal, Madrid, Spain

**Background:** Non-alcoholic fatty liver disease (NAFLD) is one of the major non-AIDS defining conditions in people with HIV (PWH), however, the diagnosis is not simple and routine screening for this condition is not well incorporated in their care. Properly validated tests and easy to apply are needed in this population in order to identify people at risk of developing chronic liver disease.

Our objective was the validation of non-invasive markers for the diagnosis of NAFLD in HIV-infected patients.

**Methods:** Prospective cohort study of PWH on stable ART regimen and persistent liver enzymes elevation without known liver disease. Whole blood test, abdominal ultrasound (US), transient elastography (including CAP) and steatosis and fibrosis non-invasive markers (TyG, HSI, FLI, FIB-4 and APRI) were performed in all participants. A liver biopsy was offered to all patients and performed in those who consented. AUROC analysis was performed to estimate the diagnostic accuracy of non-invasive tests of both steatosis and fibrosis compared to liver biopsy. An algorithm with serial combination of tests was developed.

**Results:** A total of 146 patients were included: 91% men, CDC C3 stage 14.5%, HIV RNA < 50 cop/mL 100%, median (IQR) age 49 years (41-54), BMI 27 (24-30), baseline CD4+ 740 cel/µL (593-930). Metabolic syndrome was diagnosed in 41% and diabetes mellitus or impaired fasting glucose in 43%. Medium values needed to assess the relationship to HCV clearance.

**Conclusion:** We demonstrated that a combination of TyG with FLI or HSI as first tests and US or CAP as second tests had the best diagnostic performance for liver steatosis (TyG, HSI, FLI, FIB-4 and APRI) were performed in all participants. A liver biopsy was offered to all patients and performed in those who consented. AUROC analysis was performed to estimate the diagnostic accuracy of non-invasive tests of both steatosis and fibrosis compared to liver biopsy. An algorithm with serial combination of tests was developed.

**Conclusion:** In EA persons with HIV/HCV co-infection, a rare mtDNA haplogroup (~3%) was associated with HCV SC. Limitations included small sample sizes for non-EA and HIV co-infection analyses, limited mtDNA variants, and few covariates. While this finding cannot explain substantial SC variation at a population level, it does suggest mitochondrial mechanisms of SC that may be influenced by HIV co-infection. This association should be validated in other cohorts, and mechanisms explored in translational studies.

**Table**

| Non-invasive Tests | AUC (C0.5%)
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<tr>
<td></td>
<td>Sens %</td>
</tr>
<tr>
<td>US</td>
<td>0.90 (0.75-1.00)</td>
</tr>
<tr>
<td>CAP &gt;23B dB/m</td>
<td>0.94 (0.88-1.00)</td>
</tr>
<tr>
<td>FLI &gt;4.3</td>
<td>0.81 (0.58-1.00)</td>
</tr>
<tr>
<td>HSI &gt;4.3</td>
<td>0.74 (0.62-0.87)</td>
</tr>
<tr>
<td>TyG &gt;6.38</td>
<td>0.75 (0.69-100)</td>
</tr>
</tbody>
</table>

**MODELS**

1. TyG/HSI/CAP-US | 0.99 (0.97-1.00) | <0.001 | 98    | 100   | 100 | 86  | 0.02 |

2. TyG/HSI/CAP-US | 0.92 (0.77-1.00) | <0.001 | 98    | 86    | 96  | 86  | 6.89 | 0.02 |

461 SEX-SPECIFIC INDUCTION OF TRANSCRIPTIONAL RESPONSES TO HCV INFECTION

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1Johns Hopkins University, Baltimore, MD, USA, 2Massachusetts Institute of Technology, Cambridge, MA, USA

**Background:** Spontaneous clearance of hepatitis C virus (HCV) infection occurs in ~25% of people. Defining the immunologic determinants of clearance may directly affect vaccination strategies. Female sex is associated with an increased probability of spontaneous HCV clearance (adjusted HR>2 F vs M). We sought to define sex-specific transcriptional features in HCV infection to identify immune responses that may contribute to viral clearance.

**Methods:** Total RNA was extracted from cryopreserved PBMC from a cohort of HIV-negative people with injection drug use: 39 age-matched HCV+ (20M, 19F), and 30 HCV- (15M, 15F). Dual index libraries were sequenced (Illumina NovaSeq6000 paired-end, 2x50bp). Analysis of gene counts, multidimensional scaling and gene set enrichment analysis (GSEA) were done in R. Additional analysis was done with a custom gene set derived from RNaseq of in vitro GMCSF-differentiated primary human monocytes. Comparisons of gene expression were done both within and between sex and by HCV infection status.

**Results:** HCV infection status was the primary differentiating factor in gene expression profiles across all of the samples. Excluding sex chromosome encoded genes, HCV infection induced sex-specific regulation of >5000 transcripts in females and >2800 transcripts in males and ~5000 genes shared across the sexes. The top 3 hallmark pathways by GSEA (interferon alpha, interferon gamma and complement) were shared between the sexes, but there were other unique pathways significant at the FDR-c0.05 level including negative regulation of TNF-alpha signaling by NFkB detected among females but not males. Marked upregulation of MRC1 was noted in the gene count data (HCV+ v HCV- p<2e-16), with the HCV+ females having higher expression of MRC1 (p<2e-6) than males. MRC1 is not included in hallmark pathways but is markedly enriched in an in vitro model of monocyte differentiation with GMCSF conditioning. A custom GMCSF gene set showed ~3-fold enrichment (FDR-c0.05) the gene set in HCV+, with greater enrichment in females.

**Conclusion:** HCV infection markedly alters the immune transcriptome with sex-specific features, such as a marked increase in MRC1 expression, that was reproduced in vitro by stimulating monocytes with GMCSF. These data highlight sex specific features of the innate immune response to HCV; further studies are needed to assess the relationship to HCV clearance.

**SARS-CoV-2 REPLICATION IN HEPATOCYTE CELL LINES**

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1University of Cincinnati, Cincinnati, OH, USA

**Background:** A high proportion of patients with COVID-19 demonstrate liver enzyme abnormalities which have been attributed to a variety of etiologies including sepsis, coagulopathy with ischemic injury, and drug effects. We sought to determine the potential for replication and injury due to SARS-CoV-2 infection of hepatocyte cell lines.

**Methods:** SARS-CoV-2 viral stocks were obtained from ATCC and expanded in Vero E6 cells. The virus was diluted to a multiplicity of infection of 0.1 plaque forming units and placed in medium overlying confluent cells. HepG2 and
Huh7.5 hepatocyte cell lines were utilized, with Vero E6 cells (kidney) and WI-38 (lung) cell cultures serving as infection controls. For each cell line, uninfected cells were also maintained for comparison. Infection experiments were run in triplicate. Plaque assays were used to determine supernatant viral titer on days 2 through 8 post infection. Cell culture morphology was monitored by light microscopy.

**Results:** All cell lines demonstrated significant replication potential with multi-log increase in plaque-forming units by day 3 post-infection. Rapid replication was observed through day 5. This was associated with the presence of severe cell injury with loss of attachment of the monolayer, suggesting that hepatocyte cell death limited overall levels of viral replication.

**Conclusion:** Both HepG2 and Huh7.5 cell lines support active replication of SARS-CoV-2, leading to multi-log increases in viral titer. Replication in these cell lines is accompanied by severe injury leading to loss of attachment and cell death. These findings support the concept that SARS-CoV-2 infection may be associated with liver enzyme abnormalities due to acute viral-induced liver injury.

![Figure: SARS-CoV-2 virus replication by plaque assay. Cell lines were infected with SARS-CoV-2 virus at a multiplicity of infection (MOI) of 0.5 and on day 2 post-infection, plaque assays were performed and analyzed by plaque assay for virus growth kinetics: days post infection (dpi). Error bars represent standard deviation among triplicate samples.](image)

**463 RECENT TRENDS OF HEPATITIS D VIRUS INFECTION AMONG PEOPLE LIVING WITH HIV IN TAIWAN**

Shu-Yuan Ho1, Li-Hsin Su1, Yi-Ching Su1, Wen-Chun Liu1, Hsin-Yun Sun1, Wang-Huei Sheng1, Su-Min Hsieh1, Yu-Chung Chuang1, Yu-Shan Huang1, Sui-Yuan Chang1, Chin-Chung Hung1

1National Taiwan University Hospital, Taipei, Taiwan

**Background:** People living with HIV (PLWH) who have chronic hepatitis B virus (HBV) infection are at higher risk of hepatitis D virus (HDV) infection. We aimed to investigate the recent trends of incident HDV infection among PLWH who had chronic HBV infection at a university hospital in Taiwan.

**Methods:** Between 2011 and 2018, patients seeking HIV care at the National Taiwan University Hospital were included. HBV serologic markers were determined on entry into care and during follow-up. Sequentially archived blood samples collected from PLWH with chronic HBV infection were retrieved and analyzed by serologic assays for HDV infection (anti-HDVIgM, anti-HDVIgG). Blood samples were retrospectively determined on entry into care and during follow-up. Sequentially archived blood samples collected from PLWH with chronic HBV infection were retrieved and analyzed by serologic assays for HDV infection (anti-HDVIgM, anti-HDVIgG). Blood samples were retrospectively determined on entry into care and during follow-up. Sequen...
HIV REVACCINATION IN SERONEGATIVE OR SEROREVERTED PLWH AFTER PRIMARY VACCINATION

Guän-Jhou Chen¹, Hsin-Yun Sun¹, Yu-Chung Chuang¹, Wen-Chun Liu¹, Yi-Ching Su¹, Sui-Yuan Chang¹, Chien-Ching Hung¹
¹National Taiwan University Hospital, Taipei, Taiwan

Background: After primary HAV vaccination, people living with HIV (PLWH) have a lower serologic response rate and are more likely to experience loss of seroprotective antibodies during follow-up compared to HIV-negative controls. However, it is not clear as to how HAV revaccination should be administered among PLWH who are non-responders or have lost seroprotective antibodies (seroreverters) after primary vaccination.

Methods: In this open-label randomized clinical trial, we enrolled PLWH who tested negative for anti-HAV antibodies ≥4 weeks after completing primary 2-dose HAV vaccination and those who were seroreverter after having had seroreversion to primary vaccination. Stratified by the CD4 count, all subjects were randomized at 1:1 ratio to receive either 1 or 2 doses (4 weeks apart) of HAV vaccine with a block size of 4. At week 4, week 8 (only in 2-dose group), week 24, and week 48 after HAV revaccination, the levels of anti-HAV IgG were determined with the use of a semiquantitative chemiluminescence immunoassay (ARCHITECT HAVAb-IgG, Abbott, Germany). We report the interim analysis of the results at week 24.

Results: A hundred and two participants, 50 in the 2-dose and 52 in the 1-dose group, had completed follow-up at week 24. All participants were male (mean age, 38.6 years), with median CD4 of 461 cells/mm³, and plasma HIV RNA <20 copies/ml in 90.2% before revaccination. The baseline characteristics were balanced between the two groups. The serologic responses at week 24 was similar 84.0% for the 2-dose revaccination group and 78.8% for the 1-dose group (difference, 5.2%; 95% CI, -9.9%–20.2%). However, participants in the 2-dose group had a significantly higher anti-HAV IgG level, indicated by the difference, 5.2%; 95% CI, -9.9%–20.2%). However, participants in the 2-dose group had a significantly higher anti-HAV IgG level, indicated by the

Conclusion: Among PLWH who failed to mount seroresponse after primary vaccination, people living with HIV (PLWH) who have sex with men (MSM) have a lower serologic response rate and are more likely to experience loss of seroprotective antibodies during follow-up compared to HIV-negative controls.

LOW RATE OF VACCINATION AND RISK OF INCIDENT HEPATITIS A AMONG HIV-INFECTED MSM

Marta Fernandez-Fuentes¹, Anaísa Gorma-Gomez², Pilar Rincon³, Ana Fuentes³, Esther Serrano⁴, Alejandro Gonzalez-Serna⁴, Federico Garcia⁴, Luis M Real⁴, Juan A. Pineda⁴, Juan Macias⁴
¹Hospital Universitario de Valme, Seville, Spain, ²Hospital Universitario San Cecilia, Granada, Spain

Background: Periodic outbreaks of hepatitis A virus (HAV) infection in men who have sex with men (MSM) have been observed in Western countries. Low vaccination uptake in HIV-infected individuals could drive newer outbreaks of HAV. Because of this, we aimed at evaluating the incidence of and the risk factors for HAV infection in HIV-infected patients. We also assessed the rates of HAV vaccination among this population.

Methods: In this retrospective cohort study all HIV-infected patients followed at our Unit from January 2008 to December 2019 were analyzed. Patients were included if they had at least two frozen samples available collected at least 12 months apart. HAV incident cases were defined as individuals with a baseline negative and an end of follow-up positive test for serum HAV antibodies. The year of seroconversion was investigated by testing sera stored yearly.

Results: Overall, 915 patients were included, 272 (29.7%) of them were HAV IgG seronegative at baseline. Twenty-seven (9.9%) susceptible individuals became infected during the study period. Among patients with HAV incidence, 36 (59.3%) were MSM and 71 (40.7%) were non-MSM individuals (p = 0.181). Incident cases peaked in 2009, 2012, and 2017 (Figure). Multivariate analysis, with adjusted for age, sex, risk group and CDC stage, showed an independent association between incident HAV infection and MSM (adjusted odds ratio 95% confidence interval): 4.71 (1.49–14.83) (p = 0.008). Among patients HAV IgG seronegative at baseline, 104 (38%) were vaccinated against HAV along the study period. Ten (9.6%) individuals did not show detectable anti-HAV antibodies after vaccination, and one patient (1%) lost immunity against HAV after 4 years of vaccination. Four (3.8%) non-responders to vaccination showed anti-HAV seroconversion after 5 to 12 years since vaccination.

Conclusion: The incidence of HAV infection among HIV-infected population in our area remains low and stable, with periodic outbreaks involving mainly non-immunized MSM. A significant proportion of HIV-infected patients remain susceptible to HAV infection due to insufficient vaccine uptake and limited response to vaccination. Importantly, patients not responding to HAV vaccination are at risk of infection.

SUCCESSFUL PERITRANSPLANT SOFOSBUVIR-BASED DAA THERAPY IN HIV/HCV-COINFECTED SUBJECTS

Dominic Amara¹, Marion Peters¹, Shyam Kottlilii, Norah Tenault¹, Jennifer Husson¹, Shirish Huprikar², Mark S. Sulikowski³, Christine Durand², Rodney Rogers¹, Joshua Grab¹, Henry Masur⁴, Peter Stock⁴, for the STOP-CO Investigators
¹University of California San Francisco, San Francisco, CA, USA, ²University of Maryland, Baltimore, MD, USA, ³University of Southern California, Los Angeles, CA, USA, ⁴Icahn School of Medicine at Mt Sinai, New York, NY, USA

Background: Direct acting antiviral (DAA) therapy has transformed the management of human Immunodeficiency virus (HIV) and hepatitis C (HCV) infected patients with advanced liver disease. STOP-Coinfection was a multi-center, prospective and retrospective, open-label study using sofosbuvir-based DAA therapy to treat HIV/HCV coinfected participants pre- or post-liver transplant (LT).
Methods: The study included adults living with HIV and chronic HCV and end-stage liver disease pre-LT and any stage of liver disease post-LT; HCV genotypes 1, 4, 5 or 6 with pre-treatment serum HCV RNA ≥1000 IU/mL. Pre-LT participants had a baseline Child’s Pugh Turscote (CPT) score ≥ 7 and Model for End-Stage Liver Disease (MELD) ≥ 6 to ≤ 30. Endpoints were proportion of participants achieving sustained virologic response (SVR) and reversal in decompensation.

Results: 66 participants with end-stage liver disease were enrolled, 26 had hepatocellular carcinoma. 42 participants were treated pre-LT and 26 post-LT. 93% achieved SVR and DAA therapy was well tolerated. All participants completed therapy without dose reduction or transfusion; 8 required ≥2 courses of therapy. All participants had controlled HIV on ART with stable CD4 counts and occasional low detectable viral load. 28 participants experienced 41 serious adverse events during treatment. Despite HCV cure, 12 end-stage liver disease participants required subsequent LT, 7 for decompenated liver disease. In 33 of the 42 participants treated pre-LT, MELD was available pre and post DAA treatment and pre-LT. Among the 19 participants with pretreatment MELD<15, 53% had improvement in MELD (-1 to -6); 37% had worsening of MELD (2-15); 10% no change; and 26% had post DAA treatment MELD ≥15. Among those 14 participants with pretreatment MELD≥15, 64% had improvement in MELD (-1 to -30); 29% had worsening of MELD (2 to 21); 7% no change; and 43% had MELD<15. 13 participants died, 10 with decompenated liver disease post-LT and 3 post-LT. Overall, transplant free survival was 42.8% at 4 years (figure A) and post-LT survival was 87.9% at 5 years (figure B).

Conclusion: We conclude that sofosbuvir-based DAA therapy is safe and highly effective in HCV-HIV patients with decompenated liver disease and post-LT, with post-LT survival rates comparable to other indications. This removes one of the last barriers to LT in this challenging cohort of recipients and likely will increase LT-free survival.

468 PREDICTION MODEL FOR END-STAGE LIVER DISEASE AMONG PEOPLE WITH HIV IN THE NA-ACCORD

H. Nina Kim1, Robin M. Nance2, Heidi Crane3, Bridget M. Whitney4, Keni N. Althoff5, Richard Moore6, Michael J. Silverberg7, Angel Mayor8, Edward R. Cachay9, Mark Hull10, Vincent Lo Re11, Marina B. Klein12, Joseph A. Delaney13, Mari M. Kitahata1, for the North American AIDS Cohort Collaboration on Research and Design of lDEA

Background: End-stage liver disease (ESLD) is a leading cause of non-AIDS death among people living with HIV (PWH). No predictive tool for hepatic decompensation currently exists in PWH. We sought to develop a risk prediction model for ESLD in a multicenter consortium of HIV cohorts with rigorously adjudicated clinical outcomes.

Methods: PWH who received care from one of 12 US and Canadian cohorts of the North American AIDS Cohort Collaboration on Research and Design from 2000-2016 and had FIB-4≥2.54 were included. First occurrence of ascites, varical bleed, spontaneous bacterial peritonitis or hepatic encephalopathy was verified by standardized medical record review. Those with prevalent ESLD (present from cohort entry to 180 days after) were excluded. A subset of cohorts were randomly selected until ≥20% of events were achieved and set aside for testing; remainder was used for derivation. Bayesian model averaging was used to select predictors among liver and HIV biomarkers and proxy for viral load (HIV, HBV, alcohol abuse/dependence). Variables with >50% probability of being in the best fitting model were included, along with age and sex. Harrell’s C statistic was used to assess model discrimination.

Results: Of 40,106 PWH, 13,787 (34%) had FIB-4≥2.54. Among these (82% male; 54% black; mean age 48 years), 390 ESLD events were identified over a mean 3.4 years follow-up. Of these, 66/390 were post-LT, 324 were pre-LT. ESLDS events were evenly distributed across 3 cohorts (HBV, HCV and combination). ESLDS included 52% hepatitis C, 15% hepatitis B and 31% a history of alcohol. Twelve factors (Table) together predicted ESLD risk moderately well (C-statistic 0.78, 95% CI 0.76, 0.81). The model included age, sex, race/ethnicity and routinely collected laboratory values reflecting hepatic impairment (serum albumin, AST, total bilirubin, platelets) and lipid metabolism (triglycerides, HDL, total cholesterol). Chronic HBV and HCV were among these. Neither CD4 cell count nor HIV viral load (log-transformed) had >10% probability of being included in the best fitting predictive model for ESLD, however their predictive contribution was likely mediated through other selected variables. The testing subset comprised 3,173 PWH across 3 cohorts (90% male; 14% black; mean age 49 years) and yielded 112 ESLD events over a mean of 4.3 years follow-up. Our model performed well in this testing set (C-statistic 0.81, 95% CI 0.76, 0.86).

Conclusion: This model developed specifically for PWH includes readily accessible clinical parameters, appears to work well in a diverse population and may provide a convenient tool for ESLD prediction.

Table. Prediction model for end-stage liver disease including covariates with >50% probability of being in best-fitting model by Bayesian model averaging, along with age and sex

<table>
<thead>
<tr>
<th>Covariate</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.014</td>
<td>1.001</td>
<td>1.027</td>
</tr>
<tr>
<td>Female</td>
<td>0.775</td>
<td>0.578</td>
<td>1.039</td>
</tr>
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<td>Race/ethnicity (ref: White)</td>
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<tr>
<td>Black</td>
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<td>0.461</td>
<td>0.706</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.390</td>
<td>0.871</td>
<td>2.216</td>
</tr>
<tr>
<td>Other</td>
<td>0.454</td>
<td>0.245</td>
<td>0.880</td>
</tr>
<tr>
<td>Albumin, g/dL (per doubling)</td>
<td>0.091</td>
<td>0.059</td>
<td>0.144</td>
</tr>
<tr>
<td>AST, UL</td>
<td>1.001</td>
<td>1.001</td>
<td>1.002</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>1.173</td>
<td>1.086</td>
<td>1.262</td>
</tr>
<tr>
<td>Platelets, 10^4/mm^3</td>
<td>0.992</td>
<td>0.950</td>
<td>0.988</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>1.001</td>
<td>1.001</td>
<td>1.002</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>1.015</td>
<td>1.007</td>
<td>1.023</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>0.900</td>
<td>0.886</td>
<td>0.921</td>
</tr>
<tr>
<td>Chronic hepatitis B</td>
<td>1.964</td>
<td>1.468</td>
<td>2.627</td>
</tr>
<tr>
<td>Chronic hepatitis C</td>
<td>2.354</td>
<td>1.968</td>
<td>2.804</td>
</tr>
</tbody>
</table>

469 LIVER STIFFNESS–BASED STRATEGIES FOR VARICEAL BLEEDING PREDICTION AFTER HCV CURE

Anaís Corma-Gomez1, Juan Macías1, Luis Morano2, Antonio Rivero3, Francisco Tellera4, María José Rico3, Marta Santos5, Miriam Serrana6, Rosario Palacios7, Dolores Merino8, Luis M Real9, Ignacio De Los Santos1, Francisco J. Vera-Méndez2, Juan A. Pineda10, for RISP-HEP and the GEHEP 011 Study Groups

1Hospital Universitario de Valme, Sevilla, Spain, 2Complejo Hospitalario Universitario de Vigo, Vigo, Spain, 3Hospital Universitario Reina Sofia, Cordoba, Spain, 4Hospital Universitario de Puerto Real, Cadiz, Spain, 5Hospital Universitario Virgen Macarena, Sevilla, Spain, 6Hospital Universitario de Jerez, Jerez de la Frontera, Spain, 7Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria, Spain, 8Hospital Universitario Virgen de la Victoria, Malaga, Spain, 9Hospital Universitario Juan Ramón Jiménez, Huelva, Spain, 10Hospital Universitario de Valme, Sevilla, Spain, 11Hospital Universitario de La Princesa, Madrid, Spain, 12Hospital General Universitario Santa Lucia, Cartagena, Spain

Background: Liver stiffness (LS)-based strategies identify patients with low risk of developing esophageal varical bleeding (VB) episodes, in whom unnecessary upper esophagogastroduodenoscopy (UGE) screening can be safely avoided, in the setting of HCV active infection. However, data on the accuracy of these strategies for patients with or without HIV-coinfection, after sustained virological response (SVR), are scarce.

Methods: Multicenter prospective cohort study, where HCV-monoinfected patients and HIV/HCV-coinfected individuals were included if: 1) SVR with DAA-based therapy; 2) LS < 5 kPa previous to treatment; 3) LS measurement at the SVR time-point ≥ 14 kPa. Diagnostic accuracy of HEPAVIR (favorable status LS < 21 kPa), expanded Bavero VI (favorable status LS110000/umm), and
HIV cirrhosis criteria (favorable status LS 110000/mm3), at the time of SVR, was evaluated. Missed VB episodes, negative predictive values (NPV), and number of spared UGEs were specifically assessed. Sensitivity analyses by HIV-coinfected were performed.

Results: 441 patients were included, 286 (65%) coinfected with HIV. 11 (2.5%) individuals developed a VB episode after SVR. 3 (0.7%) of them, had experienced a VB episode before SVR. The incidence rate of this event in patients with no VB prior to SVR was 0.5% (95% CI 0.3-1.0) in 100-person-years. In patients without a previous VB episode, HEPARV, expanded Baveno VI and HIV cirrhosis criteria achieved NPV for first VB episode after SVR of 99.5% (97.2%-100%), 100% (97.8%-100%) and 100% (98.1%-100%) while sparing 45%, 39% and 44% of UGEs, respectively. When considering HIV-coinfected, the performance of the three criteria was similar; in HIV-monoinfected, HEPARV, expanded Baveno VI and HIV cirrhosis criteria missed no bleeding events, maintaining NPV at 100% and sparing 55%, 39% and 44% of UGEs, respectively. Among HIV/HCV-coinfected individuals, NPV for this event was 99.2% (95.4%-100%), 100% (96.5%-100%) and 100% (96.9%-100%) for HEPARV, expanded Baveno VI and HIV cirrhosis criteria, respectively. In this subset the employment of these criteria allowed to spare 44%, 38% and 43% of UGEs, correspondingly.

Conclusion: After SVR, LS-based strategies identify HCV-infected patients, with or without HIV-coinfected, with low risk of developing VB. HIV cirrhosis criteria perform the best, sparing a higher number of UGEs without missing bleeding episodes.

470 MODULATION OF GUT FLORA PROMOTES THE REGRESSION OF ANAL DYSPLASIA IN HIV+ MSM

Eugenio Nelson Cavallini1, Letizia Santinelli, Gabriella De Girolamo1, Giuseppe P. Innocenti, Claudia Pinacchio, Luigi Celani, Marco Ridolfi, Alessandro Russo, Giancarlo Ceccarelli, Mary A. Venneri, Antonio Ciardi, Carolina Scagnolari, Alessandra Pierangeli, Claudio M. Mastroiani, Gabriella D’Ettore1,1 Sapienza University of Rome, Rome, Italy

Background: Anal microbiota of HIV+ MSM is rich in Prevotella and Bacteroides, genera that are observed in women with bacterial vaginosis (BV). BV increases genital HPV infection and persistence leading to the development of dysplasia. Degradation of mucus layer, increase of pH, alteration of several cellular pathways and impairment of T cell response are some of the mechanisms underlying the ease of HPV infection during BV. Lactic acid bacilli showed protective properties against HPV infection in women, through the restoration of epithelial lining, reduction of local pH and promotion of cytotoxic activity against HPV infected cells. Oral administration of lactic bacilli promotes HPV clearance and regression of dysplasia in women's genital tract. Here we report the preliminary results of an ongoing quadruple blind, randomized, placebo controlled clinical trial on the use of oral bacteriotherapy in HIV+ MSM with anal HPV infection (ClinicalTrials.gov Identifier: NCT04099433).

Methods: 20 HIV+ MSM concluded the study to date. At baseline (T0), participants underwent anal HPV test, anal cytology and histology of high resolution anoscopy (HRA) driven biopsies. Participants were randomly assigned to a mixture of probiotics (1800 billion bacteria/day) or placebo, for 6 months. At the end of the study (T6) the same operator repeated the investigations performed at T0. Pathology, virology and statistical analysis were conducted blindly.

Results: Main characteristics of the study population and univariate outcomes are reported in Table 1. Clearance of HPV infection was defined as negative HPV swab at T6 or evidence of a different genotype in respect to T0. In the overall population, at T6 only 3 participants in the treatment arm showed negative HPV swab (p=0.039). Impression of overall improvement or worsening were blindly assessed for each participant by HRA provider at the end of the second HRA, after reviewing images from T0. The multivariate logistic regression analysis showed that exposure to bacteriotherapy increased clearance of HPV (OR 8.9, 95% CI: 1.2-71, p=0.028), increased clearance of SIL (OR 80.0, 95% CI: 4.3-1488, p<0.001), decreased persistence and worsening of SIL (OR 0.047, 95% CI: 0.004-0.552, p=0.009) as well as the onset of new SIL (OR 0.107, 95% CI: 0.014-0.84, p=0.025).

Conclusion: Although individuals randomized in the treatment arm were younger than those exposed to placebo, administration of oral bacteriotherapy significantly improved clearance of anal HPV infection and anal SIL.

471 RISK OF AND RISK FACTORS FOR COLORECTAL CANCER IN MALE VETERANS WITH CONTROLLED HIV

Elizabeth Chiao1, Jennifer R. Kramer1, Yongquan DONG1, Christine M. Hartman1, Kathryn Royse1, Peter Richardson1, Suchismita Raychaudhury1, Sarah Ahmed1, Donna L. White1, Aaron P. Thrift1, Baylor College of Medicine, Houston, TX, USA

Background: Non AIDS Defining Cancers (NADCs) are an increasing public health problem for the growing population of PWH in the U.S. As PWH age, cancers associated with aging including Colorectal Cancer (CRC) will increase. We conducted this study to determine the incidence of and risk factors for CRC among Veterans living with HIV.

Methods: This is retrospective cohort study among HIV-positive male veterans. We included Veterans aged ≥18 years living with HIV using an algorithm including CD-9 codes, laboratory and pharmacy data from CDW. Colorectal Cancers were identified using the CD-0 codes from the VA cancer registry and identifying additional cases through ICD-9/10 code searches for colon and rectal cancer codes in the CDW. Follow-up time at risk was calculated from their date of HIV diagnosis to the development of CRC, death, or 12/31/2016, whichever was earlier. We examined incidence per 100,000 PY and Cox proportional hazards regression models to determine risk factors for disease.

Results: We included 44,160 HIV-positive male patients in the primary analysis, of whom 25,088 (56.8%) were considered to have had well-controlled HIV disease. The mean age of the HIV-positive cohort was 47.3 years (standard deviation, 10.7); and was similar for the sub-cohort of patients with well-controlled HIV disease (47.9 years). The majority of the HIV-positive patients were Black (52.3%) and had a history of tobacco smoking (75.7%), and approximately half were overweight or obese at their HIV index date. The mean duration of follow-up following HIV index date from the CDW was 8.96 years (standard deviation, 5.65) for all HIV-positive patients, and 9.88 years (standard deviation, 5.48). Among the well-controlled cohort, we found that older age and nadir CD4 ≤200 remained independently associated with increased risk for CRC, while the inverse association with statin use was somewhat stronger and statistically significant (HR, 0.60; 95% CI, 0.36-1.00). Among patients with well-controlled HIV infection, diabetes was also associated with higher risk for CRC (HR, 1.57; 95% CI, 1.00-2.48). (See Table 1)

Conclusion: In this cohort study of Veterans with well-controlled HIV, in addition to older age, and diabetes, we found that lower Nadir CD4 count was associated with increased risk for Colorectal Cancer. In addition, we found that statin utilization decreased the risk for colorectal cancer in this cohort. Further research is needed to understand the role of immunosuppression in Colorectal Cancer Risk among PWH.
Lack of TLRs Activation in Anal Cells of HIV+ Men May Contribute to HPV Persistence

Letizia Santinelli1, Mirko Scordio1, Federica Frasca1, Claudia Pinacchio1, Gabriella D’Ettorre1, Eugenio Nelson Cavallari1, Leonardo Sorentino1, Paolo Gozzo1, Alessandra Pierangeli1, Mirko Scordio1, Carolina Scagnolari1, Simonetta Simone University of Rome, Rome, Italy

Background: Persistent infection with high-risk (HR) HPVs is associated with anal cancer, with a particularly high risk in HIV+ individuals. Dampered innate immunity in the cervical mucosa is a mechanism by which HR HPVs interfere with innate immune responses, contributing to viral persistence. No such studies were conducted in HPV infected anal cells; hence, we aimed to investigate Toll Like Receptors (TLR) expression to clarify the process that leads to HPV persistence in HIV+ patients.

Methods: Anal canal brushing samples were prospectively collected from patients attending a proctology clinic. Anal cells were divided into two aliquots: one for nucleic acid extraction and one for anal cytology. Detection of HPV DNA and genotyping were performed by PCR and sequencing. To characterize the expression levels of TLRs in the mucosa of HPV-infected compared with those persistently infected. No interference with innate immune responses, contributing to viral persistence. No such studies were conducted in HPV infected anal cells; hence, we aimed to investigate Toll Like Receptors (TLR) expression to clarify the process that leads to HPV persistence in HIV+ patients.

Results: 86 Caucasian HIV-infected men (median age 46 ±11 years), on long-term ART, were enrolled in this study. HPV DNA was detected in 83.7% of anal samples, with 37/72 (51.4%) being HR-HPVs. The most common genotypes were HPV 6 (26.4%) and HPV 16 (11.1%). More than half patients (57.5%) had LSIL or intraepithelial lesions (HSIL). Among WLH with anal HPV at baseline, HPV was persistent in 54% of subjects and cleared in 46% upon follow-up (median interval: 534 days). The rate of HPV persistence was similar between subjects with anal infection alone and those with anal/cervical coinfection. Anal HSIL was associated with persistent anal HPV infection, anal HPV type 16/18, but not with cervical HPV type 16/18 infection (incidence rate ratios 6.8 (p<0.01), 6.2 (p<0.001), and 1.4 (p = 0.44), respectively).

Conclusion: In WLH, anal HPV infection may be more common than cervical infection. Persistent anal HPV infection appears to be independent of cervical HPV status. Our findings challenge the theory that the cervix forms the main reservoir of HPV and further indicates that anal cancer screening is likely warranted for WLH regardless of cervical HPV status.

Table 1: Risk factors for colorectal cancer among veterans with well-controlled HIV infection

<table>
<thead>
<tr>
<th>Age at HIV diagnosis</th>
<th>CRC (n=195)</th>
<th>Colon (n=163)</th>
<th>Rectum (n=225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>40-49</td>
<td>2.03 (1.07, 3.84)</td>
<td>2.33 (1.09, 5.08)</td>
<td>2.41 (0.82, 11.9)</td>
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<tr>
<td>50-64</td>
<td>7.66 (3.05, 19.2)</td>
<td>11.63 (3.27, 43.7)</td>
<td>2.01 (0.40, 10.1)</td>
</tr>
<tr>
<td>≥65</td>
<td>17.0 (9.00, 32.0)</td>
<td>18.5 (0.51, 68.3)</td>
<td>19.2 (0.58, 103)</td>
</tr>
</tbody>
</table>

Race/Ethnicity

<table>
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<tr>
<th>Race/Ethnicity</th>
<th>CRC (n=195)</th>
<th>Colon (n=163)</th>
<th>Rectum (n=225)</th>
</tr>
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<tbody>
<tr>
<td>White</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Black</td>
<td>1.02 (0.87, 1.56)</td>
<td>1.33 (0.83, 2.15)</td>
<td>0.35 (0.13, 0.94)</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>1.44 (0.77, 2.69)</td>
<td>1.73 (0.87, 3.47)</td>
<td>0.70 (0.16, 3.08)</td>
</tr>
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</table>

Alcohol, ever

<table>
<thead>
<tr>
<th>Alcohol, ever</th>
<th>CRC (n=195)</th>
<th>Colon (n=163)</th>
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<td>1.09 (0.68, 1.76)</td>
<td>0.87 (0.34, 2.12)</td>
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Smoking, ever

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<tr>
<td>1.08 (0.66, 1.76)</td>
<td>0.82 (0.49, 1.37)</td>
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Baseline BMI

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<td>1.00 (Ref)</td>
</tr>
<tr>
<td>25-30</td>
<td>1.02 (0.66, 1.57)</td>
<td>0.96 (0.60, 1.54)</td>
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<tr>
<td>≥30</td>
<td>0.98 (0.52, 1.77)</td>
<td>0.95 (0.51, 1.48)</td>
<td>3.85 (1.25, 11.9)</td>
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Year of HIV diagnosed

<table>
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</tr>
<tr>
<td>1996-2000</td>
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<td>1.11 (0.65, 1.96)</td>
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</tr>
<tr>
<td>2001-2006</td>
<td>0.76 (0.39, 1.46)</td>
<td>0.78 (0.38, 1.61)</td>
<td>0.47 (0.09, 2.51)</td>
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</table>

Nadir CD4

<table>
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</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>≥200</td>
<td>1.75 (1.17, 2.61)</td>
<td>1.62 (1.02, 2.54)</td>
<td>2.47 (1.02, 5.95)</td>
</tr>
</tbody>
</table>

Stat, ever

<table>
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</thead>
<tbody>
<tr>
<td>0.40 (0.36, 1.06)</td>
<td>0.66 (0.37, 1.16)</td>
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</table>

Diabetes, yes

<table>
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<th>CRC (n=195)</th>
<th>Colon (n=163)</th>
<th>Rectum (n=225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.57 (1.00, 2.48)</td>
<td>1.76 (1.03, 2.91)</td>
<td>1.09 (0.38, 3.11)</td>
<td></td>
</tr>
</tbody>
</table>

*Women with well-controlled HIV disease as those on ART therapy (>2 classes) that had achieved viral suppression for at least 80% of total follow-up time. **Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.
Background: Cervical cancer is a leading cause of cancer mortality in sub-Saharan Africa, with increased risk among women living with HIV (WLWH). Improved understanding of the prevalence and distribution of high-risk human papillomavirus (hrHPV) and measures of immune dysfunction among WLWH and cervical abnormalities despite antiretroviral therapy (ART) is required to inform regionally appropriate cervical cancer prevention strategies.

Methods: We developed a prospective cohort (2017-2020) of Ugandan WLWH on ART and HIV-seronegative women with abnormalities noted on cervical cancer screening with visual inspection with acetic acid (VIA). Those with VIA exams underwent cervical biopsies for histopathologic grading. Cervical brush specimens were tested for hrHPV DNA genotypes. Among WLWH, we further evaluated association between immune, HIV treatment-related, and social risk factors and hrHPV and cervical high grade squamous intraepithelial lesions (HSIL) findings. Associations were evaluated with Poisson regression to estimate adjusted hrHPV prevalence ratios (aPR) with robust 95% confidence intervals (95%CI).

Results: Of 16,380 women screened, 815 (5%) were VIA+, and 304 (188 WLWH, 116 HIV-seronegative) had VIA abnormalities large enough for biopsy and consented to participate. Among WLWH, median age was 34 years, ART duration 6 years, CD4 667 cells/μL, and CD4/CD8 ratio 0.9. hrHPV was detected in 67% of WLWH and 45% of HIV-seronegative women (aPR [95%CI], 1.58 [1.25, 2.00]). WLWH had increased detection of multiple hrHPV (aPR [95%CI], 3.10 [1.19, 8.08]), ≥1 hrHPV covered by the nonavalent but not quadrivalent vaccine (aPR [95%CI], 2.0 [1.1, 3.6]), and ≥1 hrHPV not covered by any vaccine (aPR [95%CI], 1.8 [1.0, 3.3]). In multivariable analyses among WLWH, lower CD4/CD8 ratio was associated with any hrHPV, multiple hrHPV, and hrHPV in the nonvalent vaccine only (Figure). 29% WLWH vs 9% HIV-seronegative women had HSIL. HIV viral load 200-1000 copies/mL was associated with HSIL cervical dysplasia.

Conclusion: WLWH with cervical abnormalities are at increased risk for prevalent single and multiple hrHPV. Risk is associated with measures of immune dysfunction despite long-term ART. HPV vaccination with the prevalent single and multiple hrHPV. Risk is associated with measures of immune dysfunction despite long-term ART. HPV vaccination with the prevalent single and multiple hrHPV. Risk is associated with measures of immune dysfunction despite long-term ART. HPV vaccination with the prevalent single and multiple hrHPV.
blocks of women living with HIV (WLWH) and HIV-negative women, matched for country of birth (1:2), were retrieved from bio-banks and HPV genotyped. 116 WLWH and 226 HIV negative women were included in the final study population. Adjusted odds ratios (adjOR), stratified by country of birth, were calculated using Conditional logistic regression.

Results: The most common HPV-types pre and post-treatment in WLWH with treatment failure were HPV16 and HPV35. The absolute risk of treatment failure in women with pre-treatment HPV16/18 was 26% (95% CI 14–44) in WLWH and 12% in HIV-negative women (95% CI 7–19), with no statistically significant difference by HIV status in conditional regression analysis (adjusted OR [adjOR] 3.2, 95% CI 0.8–12). The absolute risk of treatment failure in women with pre-treatment non-HPV16/18 was 20% (95% CI 12–31) in WLWH and 5% in HIV-negative women (95% CI 2–11). WLWH with pre-treatment non-HPV16/18 were eight times more likely to have treatment failure than HIV-negative women (adjOR 7.9, 95% CI 2.1–30.5).

Conclusion: HPV16 and 35 were the most common types in WLWH with treatment failure. WLWH with pre-treatment non-HPV16/18 were eight times more likely to have treatment failure than HIV-negative women. This could have implications for surveillance strategies following CIN2+ treatment in WLWH.

477 CERVICAL CANCER PREVENTION DURING COVID-19 PANDEMIC: THE CRS EpiC3-90 PROJECT, ZAMBIA

Mwate J. Chaila, Petronella Lumbala, Memory Kachimbe, Martin Phiri, Bosco Mukanyimzi, Linda Mwila Chibesa, Miriam Selisho, Guaya Siamalambwa, Albert Mwango, Mwaya Job Kaziadi

Catholic Relief Services, Lusaka, Zambia; 1Lusaka Provincial Health Office, Ministry of Health, Lusaka, Zambia

Background: The EPIC Control 90-90-90 (Epic 3-90) Project is a U.S. Centers for Disease Control & Prevention (CDC) funded project that supports the Ministry of Health (MoH) in Zambia to achieve the UNAIDS 90-90-90 goals in faith-based and Government facilities. The project also supports prevention activities including cervical cancer (CaCx) screening in Women Living with HIV (WLWH). CaCx remains the most frequent cancer in Zambia accounting for about 25% of all new cancer cases annually. It is also the most common cause of cancer-related death in the country. Epic 3-90 carried out technical support to the 15 supported districts from April 2020 to September 2020 to improve the CaCx screening in WLWH.

Methods: Epic 3-90 created an HIV prevention & comorbidities unit, with adequate staffing to support the establishment of CaCx screening points in supported regions. Three new screening sites were opened in locations with the largest projected number of WLHIV and 43 new providers were trained to staff both new and existing facilities. Community outreach was also carried out in all supported districts. To prevent overcrowding, in view of the COVID-19 pandemic, a staggered appointment system was employed at the sites. Other measures put in place was observance of social distancing, hand washing facilities and ensuring that both the staff and clients were masked-up. Mentorship in documentation and technical support was provided. We present findings from October 2019 to September 2020.

Results: The average number of WLHIV screened for cervical cancer increased from 242 per month (October 2019 - March 2020) to 442 (April - September 2020) indicating 82.6% improvement. This translated to an increase in the number of WLHIV who were screened from 1,450 in the first half of the year to 2,651 in the second half of the year. By September 2020, 4,101 WLHIV had been screened for CaCx (Figure 1).

Conclusion: It is possible to improve access to safe cervical cancer prevention services during the COVID-19 pandemic in WLHIV through a coordinated approach among key stakeholders and service providers. CaCx prevention services need to continue due to its high disease burden in Zambia.

478 TREATMENT TOXICITY FOR LUNG CANCER PATIENTS WITH AND WITHOUT HIV INFECTION

Keith Sigel, Maria Rodriguez-Barradas, Kimberly Stone, Matthew Goetz, Sheldon Brown, Roger Bedimo, Fatma Sheibl, Kristina Crothers, Deborah Marshall, Lesley S. Park

1John School of Medicine at Mt Sinai, New York, NY, USA; 2Michael E. DeBakey VA Medical Center, Houston, TX, USA; 3VA Greater Los Angeles Health Care System, Los Angeles, CA, USA; 4James J. Peters VA Medical Center, Bronx, NY, USA; 5VA North Texas Health Care Center, Dallas, TX, USA; 6Massachusetts General Hospital, Boston, MA, USA; 7Puget Sound VA Medical Center, Seattle, WA, USA; 8Stanford University, Stanford, CA, USA

Background: Lung cancer is the most common cause of cancer death for people with HIV (PWH). Chemotherapy and radiotherapy (RT) are frequently used for lung cancer treatment, but the risk of toxicity from these treatments is unclear for PWH.

Methods: We identified lung cancer patients (stage I-III) diagnosed 1998-2017 who received either chemotherapy or RT, from the Veterans Aging Cohort Study using linked cancer registry data. Information on demographics, comorbidity burden, HIV-related biomarkers, VACS index version 2 (V2), first-line cancer treatment and toxicity (using primary diagnosis codes associated with inpatient hospitalizations in the six-month period after chemotherapy and/or RT initiation) were collected from electronic medical record information. We compared characteristics and risk of nine chemotherapy and three RT toxicities, as well as longitudinal white blood cell and hemoglobin levels, by HIV status. We then evaluated predictors of chemotherapy adverse events for PWH by fitting a multivariable logistic model and generating predicted probabilities of toxicity.

Results: We identified 263 PWH and 477 uninfected Veterans with lung cancer receiving chemotherapy or RT. Patients were similar by HIV status according to demographics, lung cancer stage and treatment (all p>0.05). More than one-third of patients experienced a toxicity but there were no significant differences in the proportions of hospitalization for chemotherapy or RT toxicity by HIV status. Chemotherapy patients experienced substantial declines in hemoglobin and white blood cell levels during treatment, but the magnitude also did not differ by HIV status. The median decrease in CD4 count for PWH was 61%, but degree of decline was not associated with risk of toxicity or overall survival. Among PWH, both VACS index V2 (odds ratio [OR]: 1.38; p=0.01) and Charlson comorbidity score (OR 1.38 (p=0.008) were independently associated with chemotherapy toxicity risk after adjusting for age, race, and cancer stage. Predicted probabilities of major adverse chemotherapy outcomes ranged from 6.7% for the lowest VACS index and Charlson comorbidity scores, to 99% for the highest scores.

Conclusion: Patients with HIV and lung cancer were not at higher risk of chemotherapy or radiotherapy toxicity compared to uninfected patients. Comorbidity burden and VACS Index scores both predicted major chemotherapy toxicity for lung cancer patients with HIV.
Table: Chemotherapy and radiotherapy major adverse events following lung cancer treatment by HIV status.

<table>
<thead>
<tr>
<th>Adverse Outcome</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV infected</strong></td>
<td><strong>HIV uninfected</strong></td>
<td><strong>HIV infected</strong></td>
</tr>
<tr>
<td>Severe anemia, n (%)</td>
<td>23 (13)</td>
<td>51 (14)</td>
</tr>
<tr>
<td>Bacteremia, n (%)</td>
<td>2 (1)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Bronchitis, n (%)</td>
<td>4 (2)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>Cellulitis, n (%)</td>
<td>1 (1)</td>
<td>10 (3)</td>
</tr>
<tr>
<td>Dehydration/Electrolyte imbalance, n (%)</td>
<td>15 (8)</td>
<td>43 (12)</td>
</tr>
<tr>
<td>Nausea/Vomiting, n (%)</td>
<td>6 (3)</td>
<td>17 (5)</td>
</tr>
<tr>
<td>Pneumonia, n (%)</td>
<td>14 (8)</td>
<td>27 (9)</td>
</tr>
<tr>
<td>Acute renal failure, n (%)</td>
<td>7 (4)</td>
<td>25 (7)</td>
</tr>
<tr>
<td>Seizis, n (%)</td>
<td>5 (2)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Any adverse outcome, n (%)</td>
<td>64 (35)</td>
<td>126 (35)</td>
</tr>
<tr>
<td>Median relative WBC decrease after chemotherapy (IOR)</td>
<td>67% (25%-78%)</td>
<td>68% (46%-78%)</td>
</tr>
<tr>
<td>Median relative HGB decrease after chemotherapy (IOR)</td>
<td>25% (16%-34%)</td>
<td>25% (16%-35%)</td>
</tr>
<tr>
<td>Median relative CD4 decrease after chemotherapy (IOR)</td>
<td>61% (33%-76%)</td>
<td>66% (32%-78%)</td>
</tr>
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</table>
| **PHASE 1 STUDY OF LENALIDOMIDE WITH RITUXIMAB IN PRIMARY EFFUSION LYMPHOMA**

Kathryn Lurain, Ramya Ramaswami, Anaida Widell, Irene Ekedwe, Ralph Mangusani, Jomy George, Elaine S. Jaffe, Stefania Pittaluga, Maryalice Stetler-Stevenson, Hao-Wei Wang, Mark J. Roth, Vickie A. Marshall, Denise Whitby, Thomas S. Udlicki, Robert Yarchoan

1National Cancer Institute, Bethesda, MD, USA, 2National Institutes of Health, Bethesda, MD, USA, 3Frederick National Laboratory for Cancer Research, Frederick, MD, USA

**Background:** Primary effusion lymphoma (PEL) is an aggressive B-cell lymphoma strongly associated with HIV that presents as malignant effusions but also as extracavitary masses. It is caused by Kaposi sarcoma herpesvirus (KSHV), which also causes Kaposi sarcoma (KS) and multicentric Castleman disease (MCD). Prognosis is poor with survival of 10-22 months. Lenalidomide (LEN), an immunomodulatory drug, downregulates IRF4, which is overexpressed in PEL. Etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone and rituximab (EPOCH-R) is safe and effective for CD20+ HIV-associated lymphomas. While PEL is CD20-negative, rituximab eradicates KSHV-infected B-cells, a source of inflammatory cytokines, and is standard treatment for MCD.

**Methods:** In a prospective phase 1 study, we evaluated EPOCH-R and LEN (EPOCH-R2) in untreated PEL for safety. PEL was diagnosed via cytology, flow cytometry and/or biopsy. Participants (pts) received EPOCH-R days 1-5 and LEN 25 mg orally days 1-10 (with dose de-escalation for toxicity) every 21 days for 6 cycles. Pts with leptomeningeal PEL (CSF-PEL) received intrathecal prophylaxis. Pts with leptomeningeal PEL (CSF-PEL) received intrathecal prophylaxis. Pts received etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone and rituximab (EPOCH-R) is safe and effective for CD20+ HIV-associated lymphomas. While PEL is CD20-negative, rituximab eradicates KSHV-infected B-cells, a source of inflammatory cytokines, and is standard treatment for MCD. Prognosis is poor with survival of 10-22 months. Lenalidomide (LEN), an immunomodulatory drug, downregulates IRF4, which is overexpressed in PEL. Etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone and rituximab (EPOCH-R) is safe and effective for CD20+ HIV-associated lymphomas. While PEL is CD20-negative, rituximab eradicates KSHV-infected B-cells, a source of inflammatory cytokines, and is standard treatment for MCD.

**Results:** 6 HIV+ cisgender men with stage 4 PEL were enrolled July 2017-August 2019. All received integrase inhibitor-based ART. 4 had concomitant KS; 1 had MCD. Median baseline CD4+ was 321 cells/µl with no significant change at end-of-treatment (p=0.46). The most common grade 3-4 AEs were expected hematologic AEs: grade 4 neutropenia (100%), leucopenia (100%), thrombocytopenia (67%) and CD4+ lymphopenia (67%). No pts developed OIs. There were no dose-limiting toxicities; LEN 25 mg is the recommended phase 2 dose. 1 pt completed only 5 cycles due to progressive disease (PD). 2 pts who completed 6 cycles died: 1 from PD and 1 from HIV-related complications 5 months after EPOCH-R2. The response rate was 50% (95% CI:11.8-88.1). 2-year overall survival was 66.7% (95% CI:19.5-90.4).

**Conclusion:** Front-line PEL treatment with EPOCH-R2 was safe with preliminary evidence of activity and good OS and will be further evaluated in the ongoing phase 2 continuation of this trial.
481 ALTERED TUMOR MICROENVIRONMENT AND REDUCED SURVIVAL IN HIV+ HEAD AND NECK CANCERS

Syim Salahuddin1, Margaret Wu1, Javier Perez Inzarriz2, Teresita Vega2, Natalia Isaева1, Kurt Schalper1, Wendell G. Yarbrough1, Brinda Eму1
1Yale University, New Haven, CT, USA, 2Yale New Haven Hospital, New Haven, CT, USA, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Incidence of non-AIDS-defining cancers, including head and neck cancer (HNC), is rising among people living with HIV (PLWH) in the HAART era. The following study compares demographics, clinical outcomes, and the tumor microenvironment of HIV+ and Uninfected HNC patients at a single institution.

Methods: Yale Tumor Registry query identified 3,488 HNC patients, including 50 with HIV, for analysis (Clinical Cohort, 2002-2018). In addition, quantitative immunofluorescence (QIF) was performed on tumor tissue from 22 HIV+ and 75 Uninfected cases.

Results: Within our cohort, 76.0% of HIV+ and 71.9% of Uninfected patients were male, with HIV+ patients being younger (55.5 vs. 62.0, p<0.0001). Both HIV+ and Uninfected groups reported high rates of past/current tobacco use (82.0% and 71.6%, respectively) and alcohol consumption (64.0% and 61.1%, respectively). Non-Hispanic Whites comprised 82.1% of the Uninfected group, while Non-Hispanic Blacks were the most prevalent group (44.0%) among HIV+ patients, followed by Non-Hispanic Whites (40.0%) and Hispanics (16.0%). 87.9% of the HIV+ patients were on HAART, with 69.7% achieving viral loads ≤50 copies/mL and a median CD4 count of 345 cells/mm³. The majority in both groups had advanced stage (III, IV) compared to early stage (0, I, II) tumors at diagnosis (Table 1). The most prevalent HNC anatomic site among HIV+ patients was the oropharynx (OP), followed by lip/oral cavity and larynx. Among tested OP cases, 70.0% of HIV+ and 70.1% of Uninfected patients were HPV positive. There was decreased 1-yr overall survival in the HIV+ group compared to the Uninfected group (76.0 vs. 85.5%, p=0.06), which was significant among those with early stage disease (69.2% vs. 94.0%, p<0.0002). Within our tissue cohort, QIF of the tumor microenvironment revealed that HIV+ patients had lower CD8+ T cell infiltration (p=0.04), and lower PD-L1 expression in both the tumor (p=0.001) and macrophage (p=0.0002) compartments compared to Uninfected patients. No difference in PD-1 expression was noted.

Conclusion: HNC patients with HIV were significantly younger compared to the general HNC population, and experienced lower 1-yr survival with early stage disease. CD8 T cell infiltration and PD-L1 expression, which are associated with improved outcomes, appear lower among PLWH. Further evaluation of HIV-HNC subgroups, with detailed analysis of tumor site, HIV status and treatment disparities, is warranted to better delineate differences in outcome.

Table 1. Participant Baseline Characteristics, Treatment Responses, and Outcomes

<table>
<thead>
<tr>
<th>Participant</th>
<th>HIV+ (n=50)</th>
<th>Uninfected (n=3438)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>0.0%</td>
<td>78.2 (27.2%)</td>
</tr>
<tr>
<td>Stage I</td>
<td>48.0%</td>
<td>691 (20.1%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>9.18%</td>
<td>432 (12.4%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>12.0%</td>
<td>543 (15.9%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>22.44%</td>
<td>1536 (44.7%)</td>
</tr>
<tr>
<td>Stage unknown</td>
<td>4.80%</td>
<td></td>
</tr>
<tr>
<td>13.64%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatomic site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lip/Oral Cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharynx</td>
<td>3.26%</td>
<td>1230 (35.8%)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>0.0%</td>
<td>75 (2.2%)</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>3.6%</td>
<td>178 (5.2%)</td>
</tr>
<tr>
<td>Larynx</td>
<td>33.26%</td>
<td>717 (20.9%)</td>
</tr>
</tbody>
</table>

482 H3K27me3 PREVENTS ABEYANT TRANSCRIPTION AND EPISOME CLEARANCE OF KSHV

Simon Weissmann1, Adam Grundhoff2
1Heinrich Pette Institute, Hamburg, Germany

Background: Introduction: The double stranded DNA virus KSHV (Kaposi's sarcoma associated herpes virus) is the causative agent of a number of human neoplasms. It can establish lifelong chronic infections resulting in persistent reservoirs of latently infected cells. Viral latency as well as lytic reactivation of KSHV represent processes that are primarily regulated at the epigenetic level, in particular via histone modifications. Tri-methylation of histone H3 at lysine residue 27 (H3K27me3), mediated by the polycomb repressive complex 2 (PRC2), and ubiquitination of H2A lysine 119 (H2AK119Ub), mediated by the polycomb repressive complex 1 (PRC1), are associated with transcriptionally repressed chromatin and can be found on KSHV episomes early after viral entry and chromatinisation. Objectives: We want to investigate the role of H3K27me3 and H2AK119Ub in KSHV gene repression and to elucidate the impact of polycrom loss on the viral life cycle.

Methods: Here we present a cellular model for complete PRC2 depletion and investigate the effects of H3K27me3 loss in KSHV biology.

Results: Depletion of SUZ12, the structural component of the core PRC2 complex, causes a complete loss of H3K27me3 and its precursor modifications H3K27me2 and H3K27me1. Surprisingly, we observed reduced but still significant levels of H2AK119Ub, arguing that PRC1 can be recruited to KSHV independently of PRC2 activity. Furthermore, we demonstrate that loss of H3K27 methylation leads to histone hyperacetylation of KSHV episomes, supporting an active chromatin state, which ultimately leads to aberrant transcription of broad regions of the viral genome. In cells, which support KSHV's lytic lifecycle, this state of spurious transcription led to a lytic reactivation and production of viral progeny. Interestingly, when lytic reactivation was suppressed, ablation of PRC2 led to a drastic reduction of episome maintenance, resulting in increased episome loss during cell division.

Conclusion: We will discuss the potential mechanism of viral transcriptional derepression, how the absence of H3K27me3 may cause increased KSHV episome clearance, and whether increased viral transcription and episome loss are interdependent. Importantly, the availability of highly specific small molecule inhibitors against the catalytic subunit of PRC2 provides the possibility of a therapeutic intervention aiming at forced KSHV cleavage.

483 PLWH AND CANCER EXHIBIT UNIQUE PATTERN OF ACTIVATED AND EXHAUSTED CD8+ T CELLS

Omkar Chaudhary1, Diane Trotta1, Xiuping Chu2, Chip Bradley3, Yun Wang4, Jason Okulicz5, Ryan C. Maves6, Karl Kronmann7, Christina Scholfield8, Jason Blaylock9, Brian Agar10, Anuradha Ganesan11, Brinda Eму1
1Yale University, New Haven, CT, USA, 2Uniformed Services University of the Health Sciences, Bethesda, MD, USA, 3Brooke Army Medical Center, San Antonio, TX, USA, 4Naval Medical Center San Diego, San Diego, CA, USA, 5Naval Medical Center Portsmouth, Portsmouth, VA, USA, 6Madigan Army Medical Center, Tacoma, WA, USA

Background: Non-AIDS-defining cancers (NADC) are increased in incidence in people living with HIV (PLWH). We have shown that CD8+ T cells expressing PD-1 co-expressing PD-1, CD160, and CD244 are higher in PBMCs of PLWH with cancer compared to those without cancer. Expression of transcription factors (H3K27me3) loss on the viral life cycle.

Methods: 25 cancer cases (lymphoma, lung cancer and HPV-associated malignancies in those with durable viral suppression) and 87 controls were identified from the United States Military HIV Natural History Study (NHS) repository; Controls were matched for CD4+ count, duration of HIV infection and whether increased viral transcription and episome loss are interdependent. Importantly, the availability of highly specific small molecule inhibitors against the catalytic subunit of PRC2 provides the possibility of a therapeutic intervention aiming at forced KSHV cleavage.

Results: The proportion of CD8+ T cells that are T-betdimEomeshi, was higher compared to single and dual-positive subsets (p<0.0001 for all comparisons). Among subsets of cells with differential expression of inhibitory receptors,
484 TRENDS IN CANCER INCIDENCE AMONG PEOPLE LIVING WITH HIV IN ONTARIO, CANADA, 1997-2018

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Background: Since the introduction of antiretroviral therapy (ART) in the late 1980s, there has been a decrease in AIDS-related cancers (ADC), however, some jurisdictions have reported increases in non-AIDS defining cancers (NADC). We sought to confirm this finding in Ontario, Canada, a province with a high number of people living with HIV nationally and in a setting with universal access to healthcare.

Methods: We conducted a population-based retrospective cohort study of adults (~18 years) living with HIV using health administrative data including the provincial cancer registry. Participants were followed for incident cancers from January 1, 1997 to December 31, 2018. Cancers were grouped as infection-related (ADC and infection-related NADC) and infection-unrelated cancers (all other NADC). Age-standardized incidence rates (IR) per 1,000 person-years (PY) with 95% confidence intervals (CI) were calculated using direct standardization, and stratified by sex and calendar period, using the 2011 Canadian population as the reference population. The Cochran-Armitage trend test was used to examine trends across calendar periods.

Results: Of 3,454,651 PLWH, 15,059 developed cancer (overall incidence rate: 446 per 100,000 PY). The most common cancers were cervical cancer (310 cases; 18/100,000 PY), basal cell carcinoma (1,058 cases; 62/100,000 PY), Kaposi sarcoma (2,259 cases; 25/100,000 PY), and non-Hodgkin lymphoma (4,146 cases; 62/100,000 PY). The association between low CD4 cell count and higher cancer incidence was strongest for conjunctival non-Hodgkin lymphoma (1,058 cases; 62/100,000 PY), Kaposi sarcoma (2,259 cases; 25/100,000 PY), and cervical cancer (4,146 cases; 62/100,000 PY). The mechanisms through which HIV infection increases cancer risk are not well understood. We analyzed associations between immunodeficiency and cancer incidence in a nationwide cohort of people living with HIV (PLWH) in South Africa.

Methods: The South African HIV Cancer Match (SAM) Study is a nationwide cohort of PLWH in South Africa. It results from a linkage between HIV-related laboratory test records from the National Health Laboratory Services and cancer diagnoses from the National Cancer Registry for the period 2004-2014. Adults aged 18 years or over from this cohort with at least one year of follow-up after their first CD4 measurement were included. We examined associations between time-updated CD4 count and incidence rates of various infection-unrelated and infection-related cancers (further classified by underlying infectious agent). We estimated the adjusted hazard ratios (aHR) for cancer incidence per 100 CD4 cells decrease using Cox proportional hazards models. Models were adjusted for sex, age, calendar year, and comorbidity with other cancers. We assessed whether sex modified the association between CD4 cell count decrease and cancer incidence.

Results: Of 3,454,651 PLWH, 15,059 developed cancer (overall incidence rate: 446 per 100,000 PY). The most common cancers were cervical cancer (310 cases; 18/100,000 PY), basal cell carcinoma (1,058 cases; 62/100,000 PY), Kaposi sarcoma (2,259 cases; 25/100,000 PY), and non-Hodgkin lymphoma (1,058 cases; 62/100,000 PY). The association between low CD4 cell count and higher cancer incidence was strongest for conjunctival non-Hodgkin lymphoma (aHR 1.06, 95 CI 1.00-1.11), but not breast, lung, or prostate cancer. The association between low CD4 cell count and higher cancer incidence was stronger for conjunctival cancer (aHR 1.06, 95 CI 1.00-1.11), but not breast, lung, or prostate cancer. The association between low CD4 cell count and cancer incidence was strongest for conjunctival cancer (aHR 1.06, 95 CI 1.00-1.11), but not breast, lung, or prostate cancer. The association between low CD4 cell count and cancer incidence was strongest for conjunctival cancer (aHR 1.06, 95 CI 1.00-1.11), but not breast, lung, or prostate cancer. The association between low CD4 cell count and cancer incidence was strongest for conjunctival cancer (aHR 1.06, 95 CI 1.00-1.11), but not breast, lung, or prostate cancer.
486 HIGHER BURNOUT OF ONCOLOGY PROVIDERS COMPARED WITH HIV PROVIDERS IN KENYA

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Background: In sub-Saharan Africa (SSA), the burden of HIV-associated malignancies remains high despite the scale-up of antiretroviral therapy (ART). Oncology providers in SSA are limited in number and manage patients with high complexity and often poor outcomes. However, little is known about the differences in burnout among oncology providers compared to HIV providers who often co-manage patients with HIV-associated malignancies.

Methods: We approached all clinical officers and nurses working in either an oncology referral clinic or one of three representative primary care clinics for HIV medicine in the AMPATH network in western Kenya from August 2019-January 2020. Burnout was measured using the self-administered 22-item Maslach Burnout Inventory (MBI) for Medical Personnel Health Services Survey adapted for the sub-Saharan African context. MBI uses sub-scales of depersonalization (scores 0 to 30, higher indicates more burnout), personal accomplishment (scores 0 to 48, higher indicates less burnout), and emotional exhaustion (scores 0 to 54, higher indicates more burnout) (Thorsen BMC Nursing; 2011; Kim PloS 2020). Burnout was measured using the self-administered 22-item Maslach Burnout Inventory (MBI) for Medical Personnel Health Services Survey adapted for the sub-Saharan African context. MBI uses sub-scales of depersonalization (scores 0 to 30, higher indicates more burnout), personal accomplishment (scores 0 to 48, higher indicates less burnout), and emotional exhaustion (scores 0 to 54, higher indicates more burnout) (Thorsen BMC Nursing; 2011; Kim PloS One 2018). In linear regression analyses we used directed acyclic graphs to assess the determinants of burnout.

Results: Of 89 healthcare workers enrolled (23 oncology providers, 66 HIV providers), the majority (58%) were female, the median age was 37 (IQR 31-43) years and 57% were clinical officers and 43% nurses. On the depersonalization, personal accomplishment, and emotional exhaustion scales the median scores for oncology providers were 4 (IQR 3-7), 41 (IQR 32-46) and 17 (IQR 8-28) respectively, compared to 1 (IQR 0-4), 42 (IQR 39-47) and 14 (IQR 8-21) for HIV providers. After adjusting for age, gender, provider type, hours worked per week, and commute time, the mean score on the depersonalization scale for oncology providers was 2.8 units higher (p=0.006) than HIV providers, on the personal accomplishment scale 3.5 units lower for oncology providers (p=0.03) compared to HIV providers, and not significantly different for emotional exhaustion (p=0.55) (Table 1).

Conclusion: Oncology providers in Kenya had higher burnout measurements compared to HIV providers. Quantifying the factors underlying oncology provider burnout may allow health systems in SSA to identify solutions to improve provider working environments and care delivery for patients with HIV-associated malignancies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean unit difference in depersonalization scale (95% CI; p value)</th>
<th>Mean unit difference in personal accomplishment scale (95% CI; p value)</th>
<th>Mean unit difference in emotional exhaustion scale (95% CI; p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient care responsibility</td>
<td></td>
<td></td>
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<tr>
<td>HIV primary care</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Oncology</td>
<td>Ref</td>
<td>+2.8 (0.83 to 4.81; Ref</td>
<td>+0.8 (0.37 to 0.7; 0.001)</td>
</tr>
<tr>
<td>Age, per 10 year increment</td>
<td>+0.36 (0.07 to 0.45; 0.03)</td>
<td>-0.81 (-0.26 to 0.74; 0.00)</td>
<td>+0.06 (+0.01 to 0.11; 0.82)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Female</td>
<td>Ref</td>
<td>-0.58 (-1.05 to 2.75; 0.38)</td>
<td>-0.75 (-2.22 to 0.49; 0.00)</td>
</tr>
<tr>
<td>Work time per week, per 5 hour increment</td>
<td>+0.71 (+0.53 to 1.11; 0.002)</td>
<td>-0.06 (-0.13 to 0.05; 0.68)</td>
<td>+0.34 (+0.15 to 0.53; 0.00)</td>
</tr>
</tbody>
</table>

*adjHR* 0.05

487 RISK OF CANCER BY HIV STATUS AND BIRTH REGION: A NATIONWIDE REGISTER-BASED STUDY

Stina Malmström1, Philipp Wagner2, Aylin Yilmaz3, Pär Sparen1, Veronica Sudherv4, Christina Carlander4

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Background: There is a lack of register-based cancer cohort studies in people living with HIV (PLWH) that include a HIV-negative comparison group and the possibility to control for socioeconomic factors on an individual level. We aimed to examine cancer risk in the entire Swedish population categorized by HIV status, sex at birth and birth region.

Methods: The study population consisted of all people in Sweden, born 1940–2000 (n = 8 587 629), identified from the Swedish Total Population register and linked to the Swedish National HIV Register (InfCareHIV), the Swedish National Cancer Register and the Longitudinal Integration Database for Health Insurance and Labour Market Studies. The cohort was followed for three consecutive periods (1988–1997, 1998–2007, and 2008–2017). Adjusted Hazard Ratio (adjHR) of cancer (infection/non-infection-related) was calculated for each time period using Cox regression analyses, categorized by HIV-status, sex at birth and birth region, adjusted for age and income. Separate models for PLWH only were categorized by sex at birth and adjusted for age, income, birth region, nadir CD4, HIV-RNA, and mode of HIV-transmission.

Results: The proportion of cancer attributable to infection decreased over time in PLWH but was still over 40% in 2008–2017 (46% in women, 48% in men) compared to 11% in HIV-negative people. Risk of infection-related cancer was higher in PLWH than in HIV-negative people for all time periods, irrespective of sex at birth and birth region, with the highest risk seen among men in 1988–1997 (adjHR 39.0, 95% CI 33.6–45.3, ref HIV-negative men). In PLWH, infection-related cancer was associated with male sex, nadir CD4 < 200, and history of intravenous drug use (the latter for women only). There was no significant difference in risk of non-infection-related cancer by HIV status in men. Women with HIV had decreased risk of non-infection-related cancer in 1998–2007 (adjHR 0.3, 95% CI 0.1–0.7, ref HIV-negative women 1998–2007) but no significant difference was seen in the periods before or after.

Conclusion: In this nation-wide cancer-register study the risk of infection-related cancer remained higher in PLWH compared to HIV-negative people over time, irrespective of sex at birth and birth region, and was associated with level of immunosuppression and history of intravenous drug use. Early HIV detection and early antiretroviral therapy remain crucial for the prevention of cancer in this population.
ASSOCIATION BETWEEN INTEGRASE INHIBITORS (InSTIs) AND CARDIOVASCULAR DISEASE (CVD)

Bastian Neesgaard, for the RESPOND Study Group

Background: While associations between use of older antiretroviral drug (ARV) classes and CVD are well described, there are limited data related to use of InSTIs.

Methods: RESPOND participants were followed from latest of cohort enrolment or 1 Jan. 2012 (baseline) to the earliest of first CVD event (myocardial infarction [MI], stroke or invasive cardiovascular procedure [ICP]), last follow-up (FU) or 1 Oct. 2018. Logistic regression tested associations between 10-year D:A:D CVD risk score and starting an InSTI. To assess associations between CVD and InSTI exposure, multivariable negative binomial regression models were adjusted for demographics, traditional CVD risk factors, HIV-related factors, antiretroviral treatment (ART) status and concomitant or prior use of ARVs associated with CVD; where appropriate, variables were time-updated (Figure footnote).

Results: Of 21267 included individuals, 46% were exposed to one or more InSTIs during FU (2147 to raltegravir, 2385 to elvitegravir and 6372 to dolutegravir). Compared to low baseline D:A:D CVD risk odds of starting an InSTI were higher during FU (2147 to raltegravir, 2385 to elvitegravir and 6372 to dolutegravir). No other significant changes from BL to W48 were observed between pts receiving DTG+RPV and CAR. Longitudinally to W148, no significant change in C-reactive protein was observed in the ES and LS groups. Increases in the ES group in sCD14 at W48 and W100 decreased in the LS group at W100 were observed, although both groups showed significant decreases in sCD14 at W148 (P < 0.001). A similar inconsistent pattern of change was observed longitudinally in ES and LS groups for interleukin-6 and sCD163; however, pooled SWORD data showed significant increases in sCD163 at W148 in both groups (P < 0.001).

Overall, no consistent reproducible pattern of change was seen post-switch across markers (Figure). Regarding atherogenesis markers, in both SWORD studies, FABP-2 and sVCAM-1 for the ES and LS groups showed consistent significant, sustained reductions post-switch to DTG+RPV through W148. No other significant changes from BL to W48 were observed between groups receiving DTG+RPV and CAR. Longitudinally to W148, no significant change in IFN-gamma was observed, although both groups showed significant decreases in IFN-gamma at W148 (P < 0.001). A similar inconsistent pattern of change was observed longitudinally in ES and LS groups for interleukin-6 and sCD163; however, pooled SWORD data showed significant increases in sCD163 at W148 in both groups (P < 0.001).

Conclusion: In the controlled ES phase, no significant differences between arms were observed at W48 except for sCD14 favoring DTG+RPV. Longitudinally up to W148, there was no consistent, reproducible pattern of change post-switch, providing no evidence of increased inflammation or atherogenesis markers on the 2-drug regimen, DTG+RPV, while maintaining viral suppression.
QUINOLINIC ACID IS ASSOCIATED WITH CAROTID INTIMA-MEDIA THICKNESS IN HIV

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Background: Tryptophan (TRYP) metabolism via the kynurenine (KYN) pathway has been implicated in the pathogenesis of cardiovascular disease. Downstream metabolites kynurenic acid (KA) and quinolinic acid (QA) are paths for TRYP degradation that may affect differently. Inflammatory pathways mediating these effects have not been studied in HIV.

Methods: TRYP, KYN, KA and QA were quantified by LC/MS/MS from adults with and without HIV enrolled in a longitudinal study of carotid intima media thickness (IMT) progression. hsCRP, IL6, sTNF-RI and -RII, ICAM-1 and VCAM-1, D-Dimer and fibrinogen were quantified by ELISA. Data through week 96 was included. Linear mixed effects modeling with stratified propensity score matched on age, sex, race, smoking and BMI was utilized. Direct and indirect effects of inflammation markers were tested where associations between TRYP metabolites and IMT were significant.

Results: 123 adults were included (47 HIV+ initiating ART; 31 HIV+ remaining ART-naive; 45 HIV-). Median age was 40 yrs; 73% male; with BMI 27 kg/m². There were more blacks and current/former smokers in HIV+ (59 vs 27% and 68 vs 31%; both p<0.01). HIV duration (4 yrs) and HIV-1 RNA (2461 copies/ml) were similar among HIV+; but nadir CD4+ was lower in HIV+ initiating ART (340 vs 505 cells/mm³; p<0.01). There were differences in TRYP metabolites between groups at baseline and over time (Figure). Notably, in HIV+ initiating ART group, both steepness and at times direction of change in the first year after ART initiation were different from HIV+ remaining ART-naive and HIV+. Baseline QA and QA:KA ratio (trend) were associated with higher time-updated common carotid artery (CCA) IMT (p=0.05; p=0.04 and p=0.04; p=0.06), but effects attenuated with adjustment (p=0.04; p=0.07 and p=0.03; p=0.11). Baseline QA, KYN and KYN:TRYP ratio (trend) were associated with higher time-updated carotid bulb IMT even with adjustment (QA: p=0.07; p=0.04, KYN: p=0.14; p<0.05 and KYN:TRYP ratio: p=0.1; p=0.09). STNF-RII, D-Dimer and fibrinogen mediated the effect between QA and CCA IMT, while IL6, STNF-RI and fibrinogen mediated the effect between QA and bulb IMT; no mediators between KYN and bulb IMT were identified.

Conclusion: QA, but not KA, was associated with both CCA and bulb IMT, and inflammatory and coagulation markers appear to mediate these effects. Also, TRYP metabolites were associated more closely with bulb IMT, the site with the highest inflammatory milieu. ART initiation appears to impact TRYP metabolite levels.

HIW INFLAMMATORY PATHWAYS DIFER ACCORDING TO SOCIOECONOMIC INDICES IN MALAWI

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1University College Dublin, Dublin, Ireland, 2University College London, London, UK, 3Malawi-Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi, 4University of Malawi, Blantyre, Malawi, 5University of Liverpool, Liverpool, UK

Background: The role of socioeconomic (SE) indices in immune activation among PLWH is unknown, especially in low income sub-Saharan Africa (SSA), where the burden of non-communicable diseases (NCDs) is high.

Methods: Malawian adults with CD4<100 cells/ul starting ART in the REALITY trial (NCT01825031), and volunteers without HIV infection, had clinical assessment, socioeconomic evaluation, blood draw for cell surface and soluble activation markers, and carotid femoral pulse wave velocity (cfPWV) carried out at 2 and 42 weeks post-ART initiation. Linear regression models analysed SE associations with i) immune activation markers and ii) arterial stiffness.

Results: Of 279 PLWH, median (IQR) age was 36 (31-43) years and 122 (44%) were female. At baseline, T cell activation was more pronounced among those with higher SE indices. CD8 activation increased from 70% amongst those with no education to 88% amongst those with a tertiary education (p=0.002); and from 71% amongst those earning less than 10 USD/month to 87% amongst those earning between 100-150 USD/month (p=0.001, Figure 1). Adjusting for age and sex, CD8 activation and exhaustion were higher amongst those earning >25 USD/month [fold change 12.7%, p<0.001 and 6.77%, p=0.016 respectively]. CD8 activation was lower for water kiosk users [70% versus 81%, p=0.002] who also displayed lower rates of CMV PCR positivity [57% versus 57% (p<0.001)]. Conversely, monocyte activation was associated with lower SE indices. In adjusted analysis, nonclassical and intermediate monocytes were lower amongst the higher income bracket [fold change -5.23, p=0.006 and -1.91, p=0.063 respectively]. Participants with an earth floor displayed expanded nonclassical monocyte populations (p=0.04), but experienced a reduction after 42 weeks of ART [median change -2.2% versus 5.8%, p=0.026]. SE indices independently associated with arterial stiffness (adjusted for age, sex, blood pressure and haemoglobin): were: car ownership [fold change 1.3m/s (1.03 to 1.23); p=0.012]; and electricity access [1.09m/s (1.01 to 1.17); p=0.029].

Conclusion: For PLWH with high SE indices, a sedentary lifestyle combined with HIV driven T cell activation may increase vascular damage. For PLWH with low SE indices, innate immune stress – likely driven by infection, malnutrition, and poor water hygiene - is the predominant inflammatory pathway. Understanding these pathways and their drivers will help target interventions for NCDs.
ASSESSING PROTEIN BIOMARKERS' ROLE IN CVD RISK PREDICTION IN PERSONS WITH HIV (PWH)

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Background: PWH have higher rates of CVD than the general population yet CVD risk prediction models rely on traditional risk factors and fail to capture the heterogeneity of CVD risk in PWH. The purpose of this study was to identify protein biomarkers that are able to discriminate between CVD cases and controls in PWH, and to assess if a protein score can predict CVD risk beyond traditional risk factors in PWH

Methods: We analyzed 459 baseline protein expression levels from five OLINK panels in a matched CVD (MI, coronary revascularization, stroke, CVD death) case-control study with 390 PWH from INSIGHT trials (131 cases, 259 controls). We filtered out proteins that did not differentiate cases from controls (p-value >0.05); 107 proteins remained. We formed 200 training datasets via bootstrap. For each bootstrap training set, a two-component penalized logistic regression model (PLSOA) was fit. The importance of each variable in the discrimination of cases and controls in the PLSOA projection was assessed by the variable importance in projection (VIP) score. Proteins with average VIP scores > 1 were used in penalized logistic regression models with elastic net penalty, and proteins were ranked based on the number of times the protein had a nonzero coefficient. Proteins in the top 25th percentile were considered to have high discrimination. A protein score (PS) of the top-ranked proteins was developed using the bootstrap training (for weights) and the entire data.

Results: Participants had mean age 47 years, bmi 24.6 kg/m², 13% were females, 9% had CVD at baseline and 69% were on ART at baseline. Eight proteins including the hepatocyte growth factor and interleukin-6 were found to be predictive of CVD independent of established CVD and HIV factors (Odds ratio: 2.17, CI: 1.58-2.99). A model with the PS and traditional risk factors had a 5.9% improvement in AUC over the baseline model (AUCl=0.731 vs 0.69), which is an increase in model predictive power of 18%.

Conclusion: A PS improved discrimination of PWH with CVD and those without, and helped identify PWH with high risk for developing CVD. If validated, this score could be used in addition with established factors to identify CVD at-risk individuals who might benefit from aggressive risk-reduction.

ENDOTHelial MICROVESICLES: BIOMARKER & MEDIATOR OF ENDOTHelial DYSFUNCTION WITH HIV-1

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Background: Clinical interest in circulating endothelial cell derived extracellular microvesicles (EMVs) has increased due to their role in the etiology of endothelial dysfunction and the development and progression of cardiovascular disease (CVD). We have previously reported that circulating EMVs are elevated in HIV-1-seropositive adults and may contribute to endothelial dysfunction and CVD with HIV-1-infection. EMVs released under disease conditions have been shown to negatively affect endothelial nitric oxide synthase (eNOS) resulting in diminished NO bioavailability and impaired vasodilatory function. The experimental aims of this study were to determine: 1) whether circulating EMVs are associated with HIV-1-related endothelial vasodilator dysfunction; and 2) the effects of EMVs isolated from HIV-1-seropositive adults on endothelial cell NO production, in vitro.

Methods: Twenty-four sedentary, adults were studied: 12 healthy (9M/3F; 55±2 yr) and 12 HIV-1-seropositive adults on stable antiretroviral therapy (9M/3F; 53±2 yr). All subjects were non-obese, normotensive, normolipidemic, and free of overt cardiometabolic disease. Circulating EMVs (CD31+/-42b-) number was determined by flow cytometry. Forearm blood flow (FBF: via plethysmography) was assessed by intra-arterial infusion of acetylcholine and sodium nitroprusside. Human umbilical vein endothelial cells were cultured and treated with EMVs (CD144-PE) isolated from the healthy and seropositive adults.

Results: Circulating EMVs were −45% higher (P<0.05) in the HIV-1-seropositive (107±7 EMV/µL) vs healthy (74±7 EMV/µL) adults. FBF responses to acetylcholine were significantly lower (~20%) in the seropositive adults (from 4.1 ± 0.3 to 12.2 ± 0.8 mL/100 mL tissue/min vs 4.3 ± 0.4 to 15.6 ± 0.9 mL/100 mL tissue/min). EMVs were strongly and inversely associated with the vasodilator response to acetylcholine (r=-0.47; p<0.05). Expression of phosphorylated eNOS (20.9±2.3 vs 32.8±2.4 AU) and NO production (6.2±0.5 vs 9.0±0.2 μmol/L) was lower in cells treated with EMVs from the seropositive vs healthy adults. EMV-induced changes in p-eNOS and NO production in vitro were associated with the vasodilator response in vivo (r=0.58 and r=0.51; p<0.05, respectively).

Conclusion: Circulating EMVs are not only a biomarker, but also a mechanistic mediator, of endothelial dysfunction with HIV-1. EMVs represent a potential therapeutic target for improving endothelial function and thereby reducing CVD risk in HIV-1 infected adults.

CORONARY ARTERY DISEASE, TRADITIONAL RISK, AND INFLAMMATION AMONG PWH IN REPRIEVE

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Background: REPRIEVE is a large ongoing primary prevention trial of people with HIV (PWH), at risk for cardiovascular disease (CVD). The REPRIEVE Mechanistic Substudy, designed to determine unique factors contributing to CVD in PWH, assesses coronary artery disease (CAD) by coronary CTA and critical pathways of arterial inflammation and immune activation. Comprehensive data from the baseline exam establish the plaque phenotype in this key population.

Methods: The study enrolled PWH, 40-75 yrs, without known CVD, on stable ART, with low to moderate risk of atherosclerotic cardiovascular disease (ASCVD) by the 2013 ACC/AHA pooled cohort equation. Coronary CTA data were analyzed across ASCVD risk strata and in regression models including biomarkers: insulin, MCP-1, IL-6, sCD14, sCD163, LpPLA2, oxLDL, and hsCRP.

Results: Participants (n=755, 31 US sites) were 51±6 yrs, 16% female, 46% non-white, 24% Latinx with CD4 636±275 cells/mm³, and well-controlled viremia (88%), 23% vulnerable plaque (VP), and 16% high Leaman score >5 (LS). A
minority had CAC=400 (2%) or luminal stenosis ≥50% (3%). Overall plaque burden included significant non-calcified plaque. Extent of CAD increased with ASCVD risk (Figure) but plaque (30%) and VP (13%) were seen even among participants with ASCVD risk <2.5%. MCP-1, IL-6, LpPLA2, and oxLDL were higher in those with plaque. hsCRP was higher in those with VP and LS >5. In fully adjusted modeling, including ASCVD risk score, significant associations were: 1) LpPLA2 with presence of plaque, CAC=0 and LS >5, 2) MCP-1 and IL-6 with presence of plaque, 3) hsCRP with LS>5. HIV indices were generally not significant in modeling. In subgroup analyses (< or ≥75% ASCVD risk), LpPLA2 associated with CAD in those with lower risk. In contrast, oxLDL and hsCRP most consistently associated with CAD in those with higher risk, with significant interaction terms.

**Conclusion:** In this primary prevention cohort with well-controlled HIV, we demonstrate a unique CAD phenotype, including a high prevalence of non-calcified non-obstructive and vulnerable plaque, associated with increased immune activation and arterial inflammation, independent of ASCVD risk. These data suggest the importance of developing tailored CVD prevention strategies targeting inflammation and immune activation in addition to traditional risk in this population.

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**495 TREATED HIV INFECTION AND PROGRESSION OF CAROTID ATHEROSCLEROSIS IN RURAL UGANDA**

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**Background:** Studies from the United States have demonstrated HIV to contribute a unique CAD phenotype, including a high prevalence of non-calcified non-obstructive and vulnerable plaque, associated with increased immune activation and arterial inflammation, independent of ASCVD risk. These data suggest the importance of developing tailored CVD prevention strategies targeting inflammation and immune activation in addition to traditional risk in this population.

**Methods:** We conducted a prospective observational cohort study of PLWH age 40-75 with low-to-moderate traditional CVD risk. Among a subset of REPRIEVE participants, baseline levels of oxidized LDL (oxLDL) and soluble CD14 (sCD14) were characterized by ELISA. Multivariable linear regression was used to examine levels of oxLDL and sCD14, for levels of LDL cholesterol (oxLDL only), and sensitivity analyses restricted to sCD14. Findings were similar in sex-stratified analyses, analyses adjusted for sCD14. Findings were also apparent for levels of LDL cholesterol (oxLDL only), and sensitivity analyses restricted to participants with undetectable HIV-1 RNA.

**Results:** We enrolled 155 PLWH and 154 HIV-uninfected individuals and collected 5,900 cIMT images during 1,045 visits, during a median of 4 annual visits per participant (IQR 3-4, range 1-5). Age (median 50.9 years) and sex (49% female) were similar by HIV status, but PLWH had a lower systolic blood pressure compared to HIV-uninfected individuals (mean 113 vs 118, P<0.001), and were less likely to be current smokers (6 vs 21%, P<0.001), conferring a lower 10-year Framingham risk score (median 4.5 vs 6.1%, P=0.02). However, PLWH had higher high-sensitivity C-reactive protein (hsCRP) and soluble CD14 (sCD14) (P<0.001). At enrollment, PLWH had similar mean cIMT (0.665 versus 0.680 mm, P=0.15). In multivariable models, increasing age, blood pressure, and non-HDL cholesterol were associated with greater cIMT (P<0.05), however change in cIMT per year was similar by HIV serostatus (0.004 mm/year for HIV-negative [95% CI 0.001-0.007 mm], 0.006 mm/year for PLWH [95% CI 0.003-0.008 mm], HIV-time interaction P=0.25). Model estimates were similar after adjustment for inflammatory markers. In models restricted to PLWH, use of a protease-inhibitor based regimen was associated with 0.042 greater cIMT (95%CI 0.002-0.082 mm, P=0.04).

**Conclusion:** In rural Uganda, treated HIV infection was not associated with faster cIMT progression. These results do not support classification of treated HIV infection as a risk factor for subclinical atherosclerosis progression in rural sub-Saharan Africa.

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**496 FACTORS ASSOCIATED WITH SYSTEMIC IMMUNE ACTIVATION IN A GLOBAL HIV COHORT**

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**Background:** Among antiretroviral therapy (ART)-treated people with HIV (PWH), persistent systemic immune activation may contribute to atherogenesis, cardiovascular disease (CVD) events, and mortality. Factors associated with key indices of systemic immune activation have not previously been characterized among a global cohort of PWH.

**Methods:** REPRIEVE (Randomized Trial to Prevent Vascular Events in HIV) enrolled ART-treated PWH age 40-75 with low-to-moderate traditional CVD risk. Among a subset of REPRIEVE participants, baseline levels of oxidized LDL cholesterol (oxLDL) and soluble CD14 (sCD14) were characterized by ELISA. Multivariable linear regression was used to examine levels of oxLDL and sCD14 by natal sex, geographic region, and age and to evaluate other participant characteristics associated with each marker.

**Results:** The cohort included 4694 participants from 5 Global Burden of Disease regions (39% female sex, 48% Black). Median (Q1, Q3) age was 50 (45, 54) years, body mass index (BMI) 26 (23, 30) kg/m^2, LDL cholesterol 107 (87, 129) mg/dL, and CD4 count 634 (464, 842) cell/mm^3; 48% had >10 years ART exposure. Median oxLDL and sCD14 for the cohort were 53 U/L (42, 68) and 1743 ng/mL (1469, 2064). Controlling for age and sex, oxLDL was highest in high-income regions, followed by Latin America and South East/East Asia. Aside from regional variation, male sex, younger age, white race (in high-income regions), and higher BMI and waist circumference were associated with higher oxLDL and sCD4, however change in cIMT per year was similar by HIV serostatus (0.004 mm/year for HIV-negative [95% CI 0.001-0.007 mm], 0.006 mm/year for PLWH [95% CI 0.003-0.008 mm], HIV-time interaction P=0.25). Model estimates were similar after adjustment for inflammatory markers. In models restricted to PLWH, use of a protease-inhibitor based regimen was associated with 0.042 greater cIMT (95%CI 0.002-0.082 mm, P=0.04).
Conclusion: Factors associated with higher oxLDL and sCD14 – two key indices of immune-mediated CVD risk – differ. Male sex, residence in high-income regions, and higher BMI relate to higher oxLDL, while female sex, current cigarette smoking, and lower BMI relate to higher sCD14. Future studies may usefully elucidate ways in which medications and behavioral modifications (e.g., healthy diet, smoking cessation) influence oxLDL and sCD14 and whether dampening of these markers mediates CVD-protective effects.

497 SEX MODIFIES THE ASSOCIATION BETWEEN HIV AND CORONARY ARTERY DISEASE IN UGANDA

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Background: Little is known about the prevalence and risk factors for coronary artery disease (CAD) in sub-Saharan Africa, where the majority of people living with HIV (PLWH) live. We assessed the association of HIV with CAD severity and explored relationships with markers of monocyte activation in sex-stratified analyses of PLWH and people without HIV (PWOH) in Uganda.

Methods: The Ugandan Study of HIV effects on the Myocardium and Atherosclerosis (mUTIMA) follows 100 PLWH on antiretroviral therapy (ART) and 100 age- and sex-matched PWOH controls in Kampala, Uganda; all >45 years of age with >1 cardiovascular disease risk factor at study entry. At the year 2 exam (2017-2019), 189 participants had available coronary calcium (CAC) score of age with > 1 cardiovascular disease risk factor at study entry. The overall REAP Score and scores for specific diet components were generated. Higher scores indicate better diet quality. Findings were summarized by Global Burden of Disease (GBD) super-region. Adjusted linear regression analyses were performed to examine differences in diet by key covariates.

Results: Median age was 57.8 years and 63% were female. Overall, 88% had hypertension, 37% had diabetes, and 4% were current smokers. Global CAD risk was modestly higher for PWH, but not statistically significant [median (IQR) 10-year ASCVD risk 7.2% (4.0-11.8) for PLWH vs. 8.6% (4.2-16.1) for PWOH, p=0.09]. Mean duration of ART was 12.7 years and 86% had HIV viral load <50c/ml. Despite prevalent risk factors, only 19% had CAC>0 and only 21% had any detectable coronary plaque overall, without unadjusted difference by HIV status. After adjustment for ASCVD risk score, HIV status was not associated with presence of any CAD (OR 0.55, 95% CI 0.23-1.30) but was associated with presence of more severe CAD (SSS=3) among those with any CAD (OR 10.9, 95% CI 1.67-70.45). There were no differences in distribution of non-calcified vs. calcified plaque by HIV status. The prevalence of CAD differed substantially when stratified by sex and HIV status (Figure; HIV-sex interaction p=0.019).

Prevalent CAD positively correlated with classical monocytes (r=0.3, p=0.012) and negatively correlated with CX3CR1 expression (r=-0.31, p=0.011) in PLWH and negatively correlated with patrolling monocytes (r=-0.36, p=0.031) and tissue factor expression (r=-0.39, p=0.017) in PWOH.

Conclusion: Our results suggest that HIV may be associated more with progression rather than initiation of CAD in Uganda. Sex differences and unexpected inverse associations with monocyte activation markers merit further investigation.
HIV infection is independently associated with aortic aneurysms

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1Hvidovre Hospital, Hvidovre, Denmark, 2Herlev and Gentofte Hospital, Copenhagen, Denmark, 3Herlev Hospital, Hvidovre, Denmark

Background: Increased risk of cardiovascular disease has been reported in people living with HIV (PLWH). Little is known about the prevalence of aortic aneurysms among PLWH compared to uninfected individuals. Here we investigate if HIV status is independently associated with having aortic aneurysms. Furthermore, we determine risk factors associated with aortic aneurysms in PLWH.

Methods: PLWH aged 40 years or older were recruited from the Copenhagen Comorbidity in HIV Infection (COCOMO) study and matched on age and sex with uninfected controls from the Copenhagen General Population Study. Aortic dimensions were assessed using contrast enhanced CT images. Aortic aneurysms were defined according to the European Society of Cardiology; i.e. an aortic dilation at least 50% larger in diameter compared to the expected normal diameter or an infrarenal aortic diameter ≥ 30 mm.

Results: We included 594 PLWH and 1188 uninfected controls, of which 88% and 90% were male and median (IQR) age was 52 (47-60) and 52 (48-61) years, respectively. We found 46 aneurysms in 42 (7%) of PLWH and 31 aneurysms in 29 (2.4%) of the uninfected controls (p<0.001). PLWH had a significantly higher prevalence of ascending, suprarenal and infrarenal aortic aneurysms, but not abdominal aortic aneurysms (p<0.001). PLWH had increased odds of aortic aneurysms compared to uninfected controls from the Copenhagen General Population Study. Comorbidity in HIV Infection (COCOMO) study and matched on age and sex.

Conclusion: Our findings suggest that increased attention of aortic aneurysms in PLWH is warranted, and further studies should be conducted to determine if screening for aortic aneurysms in PLWH is beneficial.
Methods: In this prospective, cross-sectional study, adults with and without HIV who use and do not use heroin underwent FDG-PET to compare tissue specific inflammation, aortic (target-to-background ratio or TBR), splenic and bone marrow (standardized uptake value or SUV). Least squares regression was utilized to compare means between 1) HIV+ heroin+ vs HIV+ heroin-, 2) HIV- heroin+ vs HIV- heroin-, and 3) HIV+ heroin+ vs HIV+ heroin- and test for effect modification (heroin use-by-HIV status interaction).

Results: 110 participants enrolled (23 HIV+ heroin+, 51 HIV- heroin+, 20 HIV+ heroin-, 16 HIV- heroin-). Median (IQR) age was 41 (33, 51) yrs; 74% were men. Heroin+ were more likely Hispanic regardless of race (23 vs 6%), smokers (99 vs 44%) and to have active hepatitis C (57 vs 0%) (p<0.01 for all). Among HIV+, heroin users had lower current viral load (591 vs 790 cells/mm3), but similar nadir CD4 counts (247 cells/mm3), HIV duration (12 yrs) and proportion with HIV-1 RNA <200 copies/mL (91%). Aortic TBR was 0.41 higher in HIV+ heroin+ than HIV+ heroin- (p=0.03) and 0.26 higher than HIV- heroin+ (p=0.09) which attenuated slightly with adjustment, but were still apparent (Figure). The effect of heroin use on splenic and bone marrow SUV was opposite. Splenic (bone marrow) SUV was 0.21 (0.43) lower in HIV+ heroin+ than HIV+ heroin- (p=0.05 for spleen and p<0.001 for bone marrow) and 0.13 (0.33) lower than HIV- heroin+ (p=0.13 and p<0.0001). The differences attenuated for spleen SUV with adjustment, but remained significant for bone marrow SUV. Additionally, HIV status modified the effect of heroin use on bone marrow SUV (Figure).

Conclusion: Aortic inflammation was greatest in HIV+ heroin+, but paradoxically bone marrow and spleenic (trend) activity was the least in this group suggesting complex and possibly divergent pathophysiology within these different end organs.

503 WEIGHT GAIN AFTER SWITCHING DIFFERENT INTEGRASE STRAND TRANSFER INHIBITORS (InSTIs)

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Background: InSTIs are components of all recommended initial regimens. They have been associated with weight gain both in clinical trials and observational studies. TDF/FTC treatment has been associated with less weight gain than other NRTI pairs. Little is known about how individual InSTIs contribute to weight gain in real-world clinical practice while accounting for TDF-to-TAF switch or prior TDF.

Methods: The Trio Health HIV database was used in this retrospective study. Eligible patients (pts) were ≥18 yrs, suppressed at baseline (BL) and during study period (12 mo), switched to a new InSTI regimen (Jan’15-Jun’19), with weight measurements at BL and 12±3 mo. Univariate analyses (UV) were conducted using chi-square and t-test. Multivariable analyses (MV) using binary outcomes of gain ≥3, 5, and 10% (BL to 12 mo) were conducted using log binary models adjusting for age, gender, race, BL BMI and CD4, pre-switch and post-switch drug class, individual InSTI (DTG, EVC, BIC), and TDF-to-TAF switch (sensitivity: pre-switch TDF).

Results: Of 667 PLWH (85% men) without major ECG abnormalities at baseline, 34 (5%) developed de novo major ECG abnormalities after a median of 2.3 years. At baseline, the mean age was 51, 26% were current smokers, 2% used methadone, and 29% and 31% used protease inhibitors or efavirenz, respectively. After adjustment, age (RR: 1.57 [1.08-2.28] per decade older), being underweight (RR: 5.79 [1.70-19.71]), current smoking (RR: 2.34 [1.06-5.16]), diabetes (RR:3.89 [1.72-8.80]) and protease inhibitor use (RR: 2.45 [1.27-4.74]) were associated with higher risk of de novo major ECG abnormalities. Of PLWH without prolonged QTc at baseline, 11 (1.6%) participants developed de novo prolonged QTc. Efavirenz use was associated with 4.4 [0.9, 7.9] ms increased lengthening of QTc between baseline and follow-up. Efavirenz use was not associated with a statistically significant higher risk of de novo prolonged QTc (adjusted RR: 3.01 [0.87-10.40], p=0.082).

Conclusion: Five percent of well-treated PLWH acquired de novo major ECG abnormalities after 2.3 years of follow-up, and protease inhibitor use was associated with more than twice the risk of the de novo major ECG abnormalities. Although efavirenz was associated with longer QTc intervals, the absolute difference was small, and efavirenz was not significantly associated with higher risk of prolonged QTc after adjustment.
by InSTI after controlling for BL characteristics, prior and current drug class, and TDF-to-TAF switch vs no TDF-to-TAF switch at all gain thresholds; TDF-to-TAF switches were more likely to gain weight vs non TDF-to-TAF switch [Figure]. In sensitivity analysis accounting for pre-switch TDF vs no TDF there were also no differences in gain by InSTI type. Pts with prior TDF were more likely to gain ≥3% (aRR=1.2 CI 1.1-1.4) and ≥5% (aRR=1.4 CI 1.1-1.7) but not ≥10% (aRR=1.5 CI 0.9-2.4).

Conclusion: After accounting for pt characteristics and TDF-to-TAF switch or pre-TDF switch, there was no difference in weight gain ≥3, 5, 10% among different InSTIs. Future studies evaluate the effect of different InSTIs on weight gain should control for NRTI switches and demographics.

### Table 1

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Treatment to switch</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF switch to TAF</td>
<td>1.21 (1.0, 1.41)</td>
<td>0.0064</td>
<td>1.21 (1.0, 1.41)</td>
</tr>
<tr>
<td>Prior NRTI</td>
<td>0.77 (0.69, 0.87)</td>
<td>0.0029</td>
<td>0.81 (0.73, 0.89)</td>
</tr>
<tr>
<td>Prior NRTI switch</td>
<td>0.89 (0.73, 1.10)</td>
<td>0.3168</td>
<td>0.94 (0.78, 1.14)</td>
</tr>
<tr>
<td>Baseline BMI&lt;25kg/m^2</td>
<td>1.27 (1.1, 1.56)</td>
<td>0.0089</td>
<td>1.30 (1.13, 1.49)</td>
</tr>
<tr>
<td>Baseline CD4&lt;50 cells/mm^3</td>
<td>1.20 (1.02, 1.40)</td>
<td>0.0600</td>
<td>1.21 (1.02, 1.44)</td>
</tr>
<tr>
<td>Age ≥50</td>
<td>1.34 (1.13, 1.60)</td>
<td>0.0012</td>
<td>1.35 (1.14, 1.60)</td>
</tr>
<tr>
<td>Female vs male</td>
<td>0.88 (0.82, 0.95)</td>
<td>0.0046</td>
<td>0.90 (0.83, 0.97)</td>
</tr>
<tr>
<td>BMI&lt;30 vs ≥30</td>
<td>1.11 (1.05, 1.17)</td>
<td>0.0076</td>
<td>1.11 (1.05, 1.17)</td>
</tr>
<tr>
<td>BMI=30 vs ≥30</td>
<td>1.13 (1.07, 1.20)</td>
<td>0.0014</td>
<td>1.16 (1.09, 1.24)</td>
</tr>
</tbody>
</table>

Note: Variables associated with risk of weight gain ≥3% at 12 months.

**504 WEIGHT GAIN AMONG PWH WHO SWITCH TO ART-CONTAINING InSTIs OR TAF**

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Background: ART-associated weight changes among persons with HIV (PWH) whose first InSTI exposure is via an ART switch regimen require further long-term study in routine HIV care.

Methods: We analyzed 2007-2018 medical records data of patients who were InSTI-naive and virally suppressed (VS) for ≥1 year on non-InSTI-based ART, switched ART and remained VS. Patients were prescribed InSTI- or non-InSTI-based ART for ≥6 months, had ≥2 body mass index (BMI) values in the year before switch and ≥1 after. We analyzed weight change (BMI, presuming height was stable) before and after switch using generalized linear mixed models (GLMM), and estimated relative contribution of InSTI- and TAF-containing ART to BMI gain by contrasting GLMM-estimated slopes.

Results: Among 736 persons (with 5,316 person-years of observation), 441 (60%) switched to InSTI-based ART. Proximal to time of switch, both groups were similar regarding characteristics, prior and current drug class, and ARV regimen. ≥10% weight increase occurred in 77 (8%) participants in Q4W, 15 (5%) in Q8W, and 39 (7%) in the CAR groups. Median (range) change in BMI was 0.40 (0.20, 1.25) in Q4W, 1.25 (0.20, 7.3) in Q8W, and 1.00 kg/m^2 (0.35, 4.0) in the CAR groups. ≥3% (aRR=1.2 CI 1-1.4) and ≥5% (aRR=1.4 CI 1.1-1.7) but not ≥10% (aRR=1.5 CI 0.9-2.4) were independently associated with weight gain. Prior TDF exposure was also strongly associated with higher weight gain (BMI gain by ≥3% aRR=1.5 CI 1.2-1.9, ≥5% aRR=1.5 CI 1.2-1.9, >10% aRR=1.5 CI 0.9-2.4).

Conclusion: Among VS persons who switched ART, both InSTI and TAF use were independently associated with weight gain. During the first 8 months post-switch, the rate of weight gain was greatest and mostly associated with InSTI use; after that, continued gradual weight gain was mostly associated with TAF use. These data help define the individual contribution, magnitude, and duration of effect upon weight gain of InSTI and TAF use.

**505 WEIGHT AND LIPID CHANGES IN PHASE 3 CABOTEGRAVIR AND RILPIVIRINE LONG-ACTING TRIALS**

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Background: Weight gain and metabolic alterations have been reported with integrase strand transfer inhibitor (InSTI)-based antiretroviral (ARV) regimens. Long-acting (LA) cabotegravir (CAB), an InSTI, and rilpivirine (RPV), a non-nucleoside reverse transcriptase inhibitor, constitute a highly effective 2-drug regimen administered intramuscularly monthly or every 2 months for the maintenance of virologic suppression. Weight and lipid changes over 48 weeks in adults with virologic suppression receiving CAB+RPV LA in Phase 3/3b clinical trials are presented.

Methods: Data in participants naïve to CAB+RPV LA and randomized to CAB+RPV LA every 4 weeks (Q4W), every 8 weeks (Q8W), or oral comparator ARV therapy (CAR) through 48 weeks were pooled from the ATLAS, FLAIR, and ATLAS-2M studies. Demographics and baseline characteristics were collected for each group and changes in weight, BMI, and lipids from baseline to Week 48 were analyzed.

Results: Participants’ baseline characteristics are summarized in Table 1. Median (range) change in weight from baseline to Week 48 was 1.20 kg (-2.75, 4.09) in Q4W, 1.25 kg (-1.60, 2.22) in Q8W, and 1.00 kg (-2.80, 3.90) in the CAR groups. ≥10% weight increase occurred in 77 (8%) participants in Q4W, 15 (5%) in Q8W, and 39 (7%) in the CAR groups. Median (range) change in BMI was 0.40 kg/m^2 (-9.9, 14.3) in Q4W, 0.42 kg/m^2 (-4.8, 7.3) in Q8W, and 0.35 kg/m^2 (-8.2, 13.7) in the CAR groups. 13.4% (59/440) of participants in Q4W, 14.6% (22/151) in Q8W, and 13.8% (41/298) in the CAR groups underwent an upward shift in BMI category from normal, resulting in 3.9% (30/766, Q4W), 4.1% (11/268, Q8W), and 4.7% (23/489, CAR) of participants developing clinical obesity (BMI >30 kg/m^2). There were no clinically significant changes in triglycerides; total, HDL, and LDL cholesterol; and TC/HDL ratios among the 3 treatment groups.

Conclusion: In this pooled analysis, changes in weight and lipid parameters over 48 weeks were modest and similar, respectively, in participants receiving CAB+RPV LA Q4W or Q8W compared to CAR. Since InSTI-associated weight changes have only recently emerged, collection of weight data across the CAB development program was not standardized at sites and limited metabolic data were collected. Future and ongoing studies will further characterize potential InSTI-associated weight gain and metabolic perturbations.
506 ASSESSMENT OF OBESITY AND METABOLIC PROFILE BY INTEGRASE INHIBITOR USE IN REPRIEVE

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Background: Among people with HIV (PWH), use of integrase inhibitor (InSTI)-based antiretroviral treatment (ART) has been associated with weight gain, but the health consequences of this weight gain remain unknown. Leveraging baseline data from a large, international cohort of ART-treated PWH eligible for primary cardiovascular disease (CVD) prevention, we investigated the association of InSTI vs. non-InSTI-based ART with body mass index (BMI), waist circumference (WC), fasting glucose and low-density lipoprotein (LDL).

Methods: The Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) enrolled a global cohort of ART-treated PWH, aged 40-75 years with low-to-moderate traditional CVD risk. This cross-sectional analysis examined the effect of InSTI-use (>6 months) among participants in regions where at least 5% of the enrolled population were using InSTI-based regimens (High Income and Latin America/Caribbean Global Burden of Disease super regions). Primary analyses used linear and logistic regression; secondary analyses used quantile regression to examine differences in the distribution tails. Characteristics of those with and without entry InSTI-use were balanced by inverse probability of treatment weighting (IPTW) using generalised estimating equations to account for clustering.

Results: Among 4498 enrolling in higher InSTI-use regions, 1847 were on an InSTI; 62% of the remainder were on an NNRTI, 36% on PI. Median age was 51 years (Q1-Q3: 43-60), 23% were female, 40% were Black/African American. Among participants on an InSTI, mean BMI was 28.2 kg/m² (±5.3kg/m²), and mean WC was 97.9 cm (±18.8 cm). IPTW regression suggested higher mean BMI of +1.6 kg/m² (95% CI: 1.2-1.9) and 65% higher odds of obesity (1.4-1.9) with InSTI-use compared to no InSTI-use. InSTI-use was also associated with higher WC (Table). This difference was greatest among natal females (+2.8 cm) with InSTI-use compared to without. IPTW quantile regression suggested the greatest differences with InSTI-use were in the upper tails of BMI and WC distributions (Table). Table: Pre-switch ART regimen (%)• PI/NNRTI (62%)• NNRTI-based (38%)• NRTI-based (11%)• PI-based (9%)• PI/NNRTI-based (70%)• PI/NNRTI-based (93%)• PI/NNRTI-based (100%)

Conclusion: In an IPTW cross-sectional analysis, InSTI-use was associated with higher BMI, higher odds of obesity, and higher WC, especially among natal females, but largely not associated with elevated fasting glucose or LDL. Future longitudinal analyses of REPRIEVE participants on InSTI vs non-InSTI ART will help characterize long-term cardiometabolic effects of InSTI-use among PWH.
OBSERVES IS HIGHLY PREVALENT IN PEOPLE OF AFRICAN ANCESTRY LIVING WITH HIV IN THE UK

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Background: Obesity is a global public health emergency, and people of African ancestry are disproportionately affected. Data on obesity, and associated health issues such as diabetes mellitus (DM) and hypertension (HPT), in African populations with HIV are relatively sparse. We determined the prevalence of, and factors associated with, obesity in the GEN-AFRICA cohort.

Methods: Participants were recruited from HIV clinics across England and were eligible if they self-identified as black, aged ≥18 years and willing to provide consent. Demographic and clinical data including a diagnosis of DM and HPT were obtained. Analyses were restricted to those with both parents born in the same African region (East/South/Central/West). Multivariable logistic regression was used to analyze factors (p<0.1 in univariable analysis) associated with obesity (BMI ≥30 kg/m²).

Results: A total of 2,341 individuals [mean age 48.0 [standard deviation 9.9] years, 62% female, median CD4 count 555 cell/mm³, 99% on ART, and 93.5% with HIV RNA <200 c/ml] were included. The proportion of participants currently exposed to NRTI, NNRTI, PI and INSTI was 93%, 38%, 32%, 31%, respectively. The overall prevalence of obesity was 44% (men 30% vs. women 52%) and increased with age (Figure 1). In univariable analysis, obesity was associated with diabetes, metabolic syndrome, chronic kidney disease, obesity-associated factors (risk for HIV acquisition, CD4 count, HIV RNA, and exposure to efavirenz, raltegravir, tenofovir alafenamide (TAF) and abacavir (ABC)), and smoking status, diabetes, hypertension, cardiovascular disease (CVD) and chronic kidney disease (CKD).

In multivariable analysis, age (aOR 1.07 [1.01, 1.13] per 5 years older, female gender (2.44 [2.00, 2.97]), East African ancestry [0.67 [0.53, 0.85], HIV through hypertension (0.56 [0.33, 0.96]), CD4 count cell count (1.03 [1.01, 1.04] per 50 cell increment), HPT (1.58 [1.28, 1.95]), current smoking (0.54 [0.37, 0.79]) and current abacavir use (0.79 [0.65, 0.96]) remained associated with obesity. No significant association with DM, CVD, CKD or exposure to efavirenz, TAF or integrase inhibitors was observed.

Conclusion: We report a high prevalence of obesity in African people with HIV, with older women particularly affected. Participants of East African ancestry and on ABC-containing regimens were less likely to be obese. Obesity management should be prioritized as part of medical care to people of African ancestry with HIV.
Background: The obesity epidemic has been observed in the adult general population of Latin America (24% obese), however little is known about obesity among persons with HIV+ (PWH) in Latin America (LAm). Moreover, Latino/a PWH in the US and Canada (US/C) may have different weight trajectories than those in LAm due to environmental context. We therefore assessed weight gain after antiretroviral therapy (ART) initiation and associated factors in PWH in the Americas, contrasting with LAm and US/C Latino/a PWH and non-Latino/a PWH.

Methods: We included ART-naive, PWH >18 years old enrolled at CCASAnet or NA-ACCORD sites 2000-2016, starting ART, and with weight measures before/after ART initiation. Baseline weight was that closest to ART initiation (-180 to +30 days); weights at 1 and 3 years post-ART were those closest to these time points (+/-180 days). Generalized least squares models were used to assess trends in weight by site/ethnicity. Covariates included were age, sex, year of ART initiation, ART regimen, and weight, CD4, and viral load at ART initiation.

Results: Among 60,831 PWH, 69% were US/C non-Latino/a, 8% US/C Latino/a, 9% Latino/a in LAm, and 14% Haitians. At ART initiation, 10.0% were obese (BMI≥30): 11.7% of US/C non-Latino/a, 10.9% of US/C Latino/a, 3.8% of Latino/a in LAm, and 5.3% of Haitians. At 3 years post-ART, average weight gain among men was: 2.8 kg in US/C non-Latino/a, 3.2 kg in US/C Latino/a, 6.2 kg in Latinos in LAm, and 4.3 kg in Haitians. Average weight gain among women was: 4.2 kg in US/C non-Latino/a, 3.5 kg in US/C Latino/a, 5.2 kg in Latinos in LAm, and 0.3 kg in Haitians (Figure; global p<0.01 for men and women). In LAm, Peruvian men had the greatest weight gain at 3 years, followed by Brazilians, Mexicans, and Hondurans; Brazilian women gained the most at 3 years, followed by Peruvians and Hondurans. Overall, average weight gain was steepest at 1 year post-ART. Use of PI-based regimens, higher CD4, and lower viral load at baseline were associated with lower post-ART weight gain.

Conclusion: In the Americas, PWH substantially gain weight after ART initiation. Observed post-ART weight gain trajectories were steeper for Latinos/as in LAm and Haitian men. Initial prevalence of obesity coupled with observed weight gain suggest there may be a healthy “catch-up” phenomenon among Latino/a PWH in LAm relative to the US/C, though changes in BMI must also be examined. Nutrition and healthy migrant effects may help explain these differences.

CHARACTERISTICS OF HIV+ AND HIV− PATIENTS UNDERGOING BARIATRIC SURGERY: OBEVIH STUDY

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Background: Bariatric surgery (BS) is a major strategy to manage patients with morbid obesity which needs to be comparatively evaluated in HIV+ and HIV− patients.

Methods: ObeVIH is a prospective ongoing study of HIV+ patients with BMI >35kg/m², with comorbidities, or >40kg/m² undergoing single port sleeve gastrectomy. We compared HIV+ and HIV− groups matched 1:1 on age, sex, and BMI and evaluated the impact of HIV/ART on subcutaneous (SCAT), visceral adipose tissue (VAT) and liver histology. During BS, VAT/SCAT was recovered and scored for inflammation (macrophage crown-like structure)/peri-lobular fibrosis/peri-adipocyte fibrosis and liver biopsies were scored for steatosis/inflammation/fibrosis. We report here patients’ baseline characteristics including comorbidities and cardiovascular (CV) parameters: echocardiography findings (left ventricular mass index (LVMi) and ejection fraction (LVEF), and coronary calcium score (CCS).

Results: ObeVIH enrolled a total of 40 patients: 19 HIV+ and 21 HIV− with median (IQR) ART duration: 15y (7.5-17.5), viral suppression duration: 3.5y (2-6), and CD4 count: 864/mm³, (560-1066). ART was InSTI-Based in 15 and PI/NRTI based in 6. The anthropometric, CV, adipose tissue and liver characteristics are presented in Table 1. No difference in the prevalence of sleep apnea, hypertension, diabetes, liver/bone/kidney diseases, dyslipidemia was observed. Moreover, there was no difference between groups for LVMi (p=0.506), LVEF (p=0.371), and CCS (p=1.00). In adipose tissue, the level of inflammation was lower in InSTI-treated than in HIV− (p=0.02) and non-InSTI-treated HIV+. NASH was diagnosed by SAF score in 1/14 InSTI-treated, 2/6 non-InSTI treated HIV− and 1/16 HIV−. The level of liver steatosis was lower in InSTI-treated than non-InSTI treated HIV+ (p=0.05). There was no overall difference between HIV+ and HIV− patients.

Conclusion: We report here that HIV+ and HIV− subjects undergoing BS presented a similar profile regarding baseline CV parameters and prevalence of comorbidities. However, there were differences between InSTI-and non-InSTI treated HIV+ in SCAT and liver histology. None InSTI-treated patients had inflammation in SCAT while it was present in some non-InSTI treated HIV+ and HIV− patients, which could suggest an anti-inflammatory effect of InSTI. Moreover, liver histology showed a milder profile with less steatosis in InSTI-treated than non-InSTI treated HIV+.  

**Figure. Post-ART weight gain among PWH in the Americas, 2000-2016**

- **Male**
  - US/Canadian non-Latino/a
  - Latin American Latino/a (non-Haitian)
  - Haitian

- **Female**
  - US/Canadian non-Latino/a
  - Latin American Latino/a (non-Haitian)
  - Haitian

![Graph](image-url)
512 METABOLOGIC SIGNATURES DIFFERENTIATE WOMEN WHO GAIN WEIGHT WITH INTEGRASE INHIBITORS

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Background: Integrase strand-transfer inhibitors (InSTIs) are associated with weight gain in women living with HIV (WLH). Emerging evidence suggests that InSTIs dysregulate insulin signaling, leading to insulin resistance and excess fat storage. We compared early changes in metabolic profiles after a switch to InSTIs among women who later gained or did not gain weight.

Methods: We studied virally-suppressed (<200 copies/ml) WLH from the Atlanta Women’s Integrase Hydroxy Study (WISH) that switched to an InSTI. Data from 3 visits were used: Visit 1=pree-switch, Visit 2=within 6 months of switch, Visit 3=4-13 months after Visit 2. Stored plasma samples from Visits 1 and 2 were used; Visit 1=pre-switch, Visit 2=within 6 months of switch. ANOVA would be associated with geriatric syndromes in older adults with HIV (OAH).

Results: Of 164 participants, there were 109 (66%) males, the mean age was 61 years (SD 6), 82 (52%) identified as Black, and 93% had HIV-1 viral load <200 copies/mL. Urine cfDNA was measured in 150 participants who had urine available for analysis. The geometric mean cfDNA level in urine was 2.4x108 copies/gram of urine creatinine [95%CI: 2.0x108-3.1x108]. Two thirds (67%) met criteria for a pre-frail or frail state. Mean urine cfDNA level was higher in participants who met frailty criteria for unintentional weight loss (p=0.01) by t-test [Figure 1]. Other frailty components including slow walk, weak grip, exhaustion and low physical activity did not have statistically significant differences in mean urine mtDNA level (p-values > 0.31). In a multivariable linear regression model both microalbuminuria (p<0.001) and age (p = 0.01) were associated with higher urine cfDNA values, whereas sex, diabetes, and use of angiotensin 1 converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medications did not have statistically significant differences in mean urine mtDNA level (p-values > 0.31). In a sample subset with body composition data (n=138), median body mass index was in the overweight range (median=27kg/m2, IQR 24-31). In separate multiple linear regression models adjusted for sex, microalbuminuria, higher urine cfDNA was inversely associated with skeletal muscle index (SMI) (β =-0.19, p=0.02) as well as fat mass index (FMI) (β =-0.08, p=0.02).

Conclusion: Urine cfDNA may have a role as a novel biomarker of geriatric syndromes in OAH that may feature unintentional weight loss and lower skeletal muscle and fat mass indices.

513 URINE MITOCONDRIAL DNA, WEIGHT LOSS, AND BODY COMPOSITION IN OLDER ADULTS WITH HIV

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Background: Mitochondrial DNA released from cells undergoing stress and necrosis-mediated cell death has the potential to act both as mediator and marker of inflammatory dysregulation, and to serve as a biomarker in the blood and urine. We hypothesized that urine cell-free mitochondrial DNA (cfmtDNA) would be associated with geriatric syndromes in older adults with HIV (OAH).

Methods: This is a cross sectional analysis of OAH (age 55 and over) who had frailty testing (Fried Frailty phenotype), bioelectric impedance analysis (BIA), and measurement of urine cfmtDNA by quantitative PCR. Skeletal muscle and fat mass indices were calculated using BIA results.

Results: Of 164 participants, there were 109 (66%) males, the mean age was 61 years (SD 6), 82 (52%) identified as Black, and 93% had HIV-1 viral load <200 copies/mL. Urine cfmtDNA was measured in 150 participants who had urine available for analysis. The geometric mean cfDNA level in urine was 2.4x108 copies/gram of urine creatinine [95%CI: 2.0x108-3.1x108]. Two thirds (67%) met criteria for a pre-frail or frail state. Mean urine cfDNA level was higher in participants who met frailty criteria for unintentional weight loss (p=0.01) by t-test [Figure 1]. Other frailty components including slow walk, weak grip, exhaustion and low physical activity did not have statistically significant differences in mean urine mtDNA level (p-values > 0.31). In a multivariable linear regression model both microalbuminuria (p<0.001) and age (p = 0.01) were associated with higher urine cfDNA values, whereas sex, diabetes, and use of angiotensin 1 converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medications did not have statistically significant differences in mean urine mtDNA level (p-values > 0.31). In a multivariable linear regression model both microalbuminuria (p<0.001) and age (p = 0.01) were associated with higher urine cfDNA values, whereas sex, diabetes, and use of angiotensin 1 converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medications did not have statistically significant relationships with urine mtDNA levels (p=0.16, p=0.42, p=0.44, respectively). In a sample subset with body composition data (n=138), median body mass index was in the overweight range (median=27kg/m2, IQR 24-31). In separate multiple linear regression models adjusted for age, sex and microalbuminuria, higher urine cfDNA was inversely associated with skeletal muscle index (SMI) (β =-0.19, p=0.02) as well as fat mass index (FMI) (β =-0.08, p=0.02).

Conclusion: Urine cfDNA may have a role as a novel biomarker of geriatric syndromes in OAH that may feature unintentional weight loss and lower skeletal muscle and fat mass indices.

Table 1. Pathway characterization of changes in amino acids, carbohydrates, lipids, and energy metabolism (prepared by Dr. Trang Duong). Metabolites were identified using MetaboAnalyst. A p-value <0.05 is considered statistically significant. p-values for the groups comparison were calculated by t-test.

Conclusion: Significant changes in amino acid pathways are reflective of insulin resistance and causative of body weight gain following a switch to InSTIs. Changes in bioenergetic pathways suggests altered mitochondrial utilization of fuels, or metabolic inflexibility, induced by InSTI use among those who later gain weight. Because these metabolic changes occurred soon after InSTI initiation and prior to weight change, these data provide mechanistic insight into InSTI-specific body weight changes over 1-2 years and should be validated in a larger population.
**IN VITRO MODEL TO ASSESS ANTIRETROVIRAL THERAPY ON ADIPOCYTE BIOLOGY**

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**Background:** Antiretroviral therapies (ART) have diverse effects on adipose tissue biology, clinically observed through changes in weight and fat distribution with ART initiation or switch. These effects likely occur in a fat depot-specific manner, however the mechanisms remain poorly understood. Our objective was to develop an in-vitro model which allows in-depth examination of the cellular effects of specific ART regimens on adipocyte biology.

**Methods:** We utilized five paired human preadipocyte cultures from omental (Om) and abdominal subcutaneous (Abdsc) depots from HIV uninfected individuals to examine effects of an integrase-inhibitor (InSTI); dolutegravir (DTG), compared to a protease inhibitor; Darunavir (DRV), on adipocytes. Cells were differentiated for 7 days using an adipogenic medium. After 7 days, adipocytes were switched to a maintenance media and treated with DMSO (Control) or DTG, 3.1μg/mL for 7 days. Adipocytes were then maintained on DTG (STAY) or switched to DRV, 11.8μM, (SWITCH) until day 21. Triglyceride content was assessed by enzymatic assay and normalized to DNA content. Adipogenic and fibrotic gene expression was assessed using RT-qPCR, and adipokine secretion was determined with ELISA.

**Results:** In both Om and Abdsc cells, exposure to DTG and DRV did not affect viability. Triglyceride accumulation did not differ between ART exposures. Adipocytes in the STAY condition had significantly increased expression of PPARγ, a late adipogenic marker, in both Om and Abdsc adipocytes (1.6 fold & 1.3 fold, p<0.05) and did not diminish with SWITCH. Expression of collagen-6 mRNA, a fibrotic marker, was increased in the STAY condition in Abdsc (1.4 fold, p<0.05) but not Om. Both leptin and adiponectin (ADCN) secretion were significantly decreased in the STAY condition (6.6pg leptin/ngDNA vs. 18.9pg; 1.0pg ADCN/ngDNA vs. 2.6pg, p<0.05), and this was partially ameliorated in the SWITCH condition (12.8ng leptin; 2.0pg ADCN, p<0.05) in Abdsc but not in Om adipocytes (Image).

**Conclusion:** We developed an in-vitro model using differentiated primary human adipocytes which can examine differential depot-dependent effects of specific ART on adipocyte biology. The observed decrease in leptin, a major satiety hormone, in the STAY group may help explain the increased weight gain observed in individuals taking InSTIs. This model can help define mechanisms by which ART causes adverse metabolic effects and used in pre-clinical applications to test potential, unintended adipocyte-specific effects of future ART.

**INTEGRASE INHIBITORS TARGET MITOCHONDRIA IN BROWN ADIPOCYTES DISRUPTING THERMOGENESIS**

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**Background:** Antiretroviral therapy (ART) containing integrase strand transfer inhibitors (InSTIs) has been associated with weight gain in both ART-initiation and switch studies, especially in women, but the underlying mechanisms are unclear. Estrogen promotes brown adipocyte differentiation while suppressing white adipose differentiation. Hence, we hypothesized that InSTIs may interrupt adipose function via inhibition of estrogen action.

**Methods:** Primary preadipocytes were isolated from 4 weeks female mouse (C57BL/6). Cells were treated with InSTI (dolutegravir (DTG) or bictegravir (BIC)) or doravirine (DOR) 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. Estrogen receptor mediated transcriptional activity was measured by luciferase reporter containing estrogen response element. Finally, we examined the effects of DTG (10mg/kg for 5 days) on food intake, energy expenditure, oxygen consumption in female mice using Comprehensive Laboratory Animal Monitoring System.

**Results:** We found that DTG and BIC mildly induced white adipocyte differentiation measured by white adipogenic markers (SREBP, CEBPα, and PPARγ) and lipid accumulation. In contrast, brown adipogenic markers (SREBP, PGC1α and FABP4) were significantly reduced by DTG or BIC exposure (50-80%). Uncoupling protein1 (UCP1), which is an essential for a thermogenic process in brown/beige adipocytes, was downregulated by more than 90% compared to no treatment group. In addition, a decrease in UCP1 in brown adipocytes was accompanied by a decrease in cytochrome oxidase complex IV (COX IV) in mitochondria as well as GAPDH, a key glycolytic enzyme. Moreover, estrogen receptor-reporter assay revealed that estrogen-mediated pathway was blocked by DTG by 70%. DOR had no effect on fat differentiation, UCP1 expression, or mitochondrial enzyme activity. In vivo, DTG administration to female mice inhibited oxygen consumption and energy expenditure by 15% without affecting food consumption.

**Conclusion:** In in vitro models, InSTI exposure had opposite effects on the differentiation of white and brown fat. In brown adipocytes, the inhibition of brown thermogenic function by DTG was associated with interruption of mitochondrial proteins (e.g. COX IV and UCP1), which may be mediated through estrogen receptor. These findings suggest a novel mechanism by which InSTIs may lead to weight gain, especially in women.

**INCIDENT DIABETES ASSOCIATED WITH INTEGRASE STRAND TRANSFER INHIBITOR INITIATION**

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**Background:** Integrase strand transfer inhibitors (InSTIs), in particular dolutegravir, have been associated with weight gain in people with HIV (PWH). However, limited data exists on other metabolic outcomes in PWH on this class of ART. We examined the risk of new-onset diabetes mellitus and hyperglycemia in PWH on InSTI-based regimens.
Results: 517 PREDICTED 10-YEAR RISKS OF CARDIOVASCULAR DISEASE AND DIABETES IN PERSONS WITH HIV

518 RISK FACTORS FOR PROGRESSION FROM PREDIABETES TO DIABETES IN PERSONS WITH HIV

Methods: Data from the IBM® MarketScan® databases for commercially and Medicaid insured adults were used to identify PWH on ART. The date of initiation or switch to InSTI was used as the index date for InSTI users while the date of initiation or 180 days after the first claims (for prevalent users) was used for non-InSTI users. The primary outcome was a composite of new-onset diabetes mellitus or hyperglycemia in the six months post-ART initiation. We identified outcomes and covariates associated with risk of diabetes mellitus and hyperglycemia using ICD-9-CM/ICD-10-CM diagnosis and procedure codes and CPT-4 codes. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for association between new-onset diabetes and hyperglycemia and overall or individual InSTI use.

Results: 111,652 PWH initiated ART between Jan 1, 2007 and June 30, 2018. 34,398 (31%) were treated with InSTI-based regimens (raltegravir 37.7%, elvitegravir 35.5%, dolutegravir 26.8%). Mean age was 42.3 (standard deviation 10.9) years, 78% were male, and 16% were Medicaid insured. The primary outcome occurred in 2,836 PWH (93% new-onset diabetes mellitus, 7% hyperglycemia). PWH on InSTIs were 22% more likely to develop new-onset diabetes mellitus or hyperglycemia (HR 1.22 [95% CI 1.13, 1.32]). When InSTIs were included in the model individually, PWH on dolutegravir were 47% more likely to develop new-onset diabetes mellitus or hyperglycemia (HR 1.47 [95% CI 1.33, 1.66]), while those on elvitegravir were 20% more likely (HR 1.20 [95% CI 1.01, 1.40]). No association was observed in those on raltegravir-based therapy (HR 0.94 [95% CI 0.92, 1.17]).

Conclusion: Overall, InSTI use was associated with increased risk of new-onset diabetes mellitus or hyperglycemia in the six months post index. The risk was more than twice as high in those on dolutegravir compared with those on elvitegravir while raltegravir was not associated with this finding. Further research is necessary to understand the mechanism driving these findings.
519 HYPERGLYCEMIA AND DIABETES MELLITUS IN PERSONS LIVING WITH AND WITHOUT HIV IN AFRICOS

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Background: Diabetes mellitus (DM) and hyperglycemia are prevalent in persons living with HIV (PLWH) in high-income countries and are more common with an aging cohort. Little data on their prevalence is available in Africa especially in PLWH over 50, a growing population of individuals on treatment. There are also concerns about development of hyperglycemia with antiretroviral therapy (ART), particularly with dolutegravir (DTG). We examined differences in prevalence of DM and hyperglycemia in an ongoing African Cohort study.

Methods: The African Cohort Study (AFRICOS) is a prospective cohort enrolling adults with and without HIV at 12 clinical sites in Kenya, Tanzania, Uganda and Nigeria. Data were collected from January 2013 to September 2020 evaluating prevalence and incidence of DM and hyperglycemia. DM was defined as a fasting glucose >126 mg/dL or receipt of medication for DM and hyperglycemia was defined as fasting glucose >99 mg/dL, nonfasting > 199 mg/dL or medication. Multivariable logistic regression with generalized estimating equations was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for factors associated with diabetes and hyperglycemia.

Results: There were a total of 3568 participants, 1493 (41.8%) males and 2075 (58.2%) females. PLWH comprised 2949 (82.7%), and 679 (17.3%) without HIV. 560 (15.7%) were age >50. At enrollment there was a statistically significant difference in prevalence of DM and hyperglycemia by HIV status and age. PLWH age >50 and on ART had the highest prevalence of hyperglycemia (16%), ART naïve PLWH age >50 had the highest prevalence of DM (7%), Figure). After adjustment for sex and study site, PLWH age 50+ had 5.29 the odds of having DM (95% CI: 2.61-10.70) and 86% increased odds of hyperglycemia (95% CI: 1.38-2.50) at all visits compared to people without HIV age 50. DM incidence did not vary by HIV and ART status including receipt, of DTG. Incidence of hyperglycemia was higher in PLWH <50.

Conclusion: In this large US population-based study, we demonstrate that gender is a defining factor when considering the association between HIV and T2DM. PLWH and T2DM are disproportionately affected by T2DM with early data suggesting this could be due to higher rates of obesity. HIV-related clinical guidelines on metabolic risk should ensure adequate enrollment of PLWH to take account of the differential risk for T2DM.

520 SEX DIFFERENCES IN DIABETES PREVALENCE AMONG PERSONS WITH HIV IN THE UNITED STATES

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Background: There is conflicting evidence for an association between type 2 diabetes mellitus (T2DM) and HIV. Notably, most studies of T2DM prevalence among persons with HIV (PWH) have a heavy male preponderance. We assessed gender as a factor impacting the association between T2DM and HIV in the United States.

Methods: A cross-sectional study using a multi-health system electronic medical record analytics platform was performed (Explorys; IBM Watson Health, Cambridge, MA, USA). The database contains 64 million persons, representing 15% of the population across all 4 census regions of the United States. Persons with all types of insurance including self-pay are represented. All adults active in the database between Nov 2015 and Nov 2020 with complete records on age, gender, race and body mass index were included. PWH were identified using ICD-9 or ICD-10 codes related to HIV and prescription for antiretroviral therapy. T2DM was defined using SNOMED-CT terms that corresponded to ICD 9 or 10 codes 250.x0-x2 and E11.8, respectively. Comparisons between groups were performed using the chi-square test with a P-value ≤0.05 being considered statistically significant.

Results: We identified 39,500 PWH and 13,015,560 HIV-seronegative controls. PWH were mostly younger than 60 years of age (29,260, 74%), 74% were male and 26% were female. Women with HIV (WWH) were more likely to be black and obese compared to men with HIV (MWH) (p<0.001). Prevalence of T2DM was higher among WWH compared to HIV-seronegative women (22% vs 14%, p<0.001). WWH were more likely to have T2DM across all age-subgroups (Figure 1). The prevalence of T2DM among MWH was lower compared to HIV-seronegative controls (15% vs 17%, p<0.002).

Conclusion: In this large US population-based study, we demonstrate that gender is a defining factor when considering the association between HIV and T2DM. WWH are disproportionately affected by T2DM with early data suggesting this could be due to higher rates of obesity. HIV-related clinical studies on metabolic risk should ensure adequate enrollment of WWH to take account of the differential risk for T2DM.

521 MITOCHONDRIAL DNA HAPLOGROUPS AND RISK OF DIABETES IN VETERANS WITH AND WITHOUT HIV

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Background: Type 2 diabetes mellitus (DM) is common in persons with HIV. Immune activation, senescence, and mitochondrial dysfunction may contribute to DM risk. We have previously shown that DM risk differed by mitochondrial DNA (mtDNA) haplogroups in women of African ancestry with HIV. We sought to examine haplogroup associations with DM risk and available T cell phenotypes among persons with and without HIV in the Veterans Aging Cohort Study (VACS) Biomarker Cohort.

Methods: VACS participants had DM outcomes determined by an algorithm including lab values, medications, and diagnosis codes. mtDNA haplogroups were derived from genome-wide genotyping and HaploGrep. Analyses included logistic regression of prevalent DM and Cox regression of incident DM, stratified by self-reported ancestry and HIV status. T cell phenotypes were determined from flow cytometry of cryopreserved peripheral blood mononuclear cells. Covariates in adjusted models included age, body mass index, hazardous drinking status, HCV status, CD4+ T cell count, and plasma HIV RNA level at
VACS entry, and selected T-cell phenotype markers. Prevalent DM cases were excluded from incidence analyses.

**Results:** A total of 2019 Veterans (65% with HIV) had mtDNA haplogroups, DM outcomes, and T-cell phenotyping. The majority were non-Hispanic Black (68%) and male (95%); 21% had prevalent DM and there were 202 cases of incident DM over a median of >8 years of follow-up. Among 781 Black Veterans with HIV and no prevalent DM, mtDNA haplogroup L3 (N=313) was associated with incident DM (HR 1.6; 95% CI 1.1-2.5) adjusting for the factors above and percent senescent (CD28-) CD4+ T cells, which were lower in Black Veterans having haplogroup L3 vs. other African haplogroups (median 13% vs. 15%; Wilcoxon p=0.03). No European or other African haplogroups were associated with prevalent or incident DM, and there were no statistically significant associations in Veterans without HIV.

**Conclusion:** A common mtDNA haplogroup was associated with incident DM in non-Hispanic Black Veterans with HIV. This haplogroup was also associated with DM risk in a prior study of Black women with HIV. While the VACS is large, the number without HIV and individual haplogroups were small, and incident DM cases were relatively few. Haplogroup L3 was also associated with less senescent CD4+ T cells in peripheral blood, but this may not be the primary driver of its relationship with DM. Further study is needed to characterize biologic effects of haplogroup L3 that may confer DM risk.

**522 RELATIONSHIP OF T-CELL MITOCHONDRIAL GENE EXPRESSION AND DIABETES IN PERSONS WITH HIV**

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**Background:** Metabolic diseases including type 2 diabetes mellitus (T2DM) are common in persons living with HIV (PLWH). Studies have found reduced mitochondrial DNA (mtDNA) copy number in peripheral blood mononuclear cells (PBMCs) is associated with T2DM in the general population. Single cell RNA sequencing (scRNA-seq) offers a promising method to study mtDNA expression at the cellular level. We hypothesized that PLWH and T2DM will have reduced mtDNA gene expression in T cells from PBMC and adipose tissue compared with PLWH without T2DM.

**Methods:** Four diabetic and 4 non-diabetic PLWH from a larger cohort who were on antiretroviral therapy for at least 18 months with viral suppression (<50 copies/mL) for at least 12 months underwent PBMC and subcutaneous abdominal tissue liposuction. Samples were stained and underwent flow cytometry for phenotyping and single cell sorting. Well-specific barcodes were used to generate single-cell cDNA libraries followed by 3’ and 5’ amplification. Paired-end reads were sequenced using Illumina NextSeq and reads were then aligned to the GRCh38 human reference genome. Thirteen mtDNA coding genes were identified and average gene expression was calculated and normalized as a ratio of overall gene expression. Normalized mtDNA expression in diabetics was compared with non-diabetics using the Student’s t-test in PBMC and adipose tissue separately.

**Results:** Diabetic and non-diabetic individuals did not differ significantly by age or body mass index. The average normalized mtDNA expression was lower among individuals with T2DM compared with individuals without T2DM in CD4+ (16.8 vs 19.5; p<0.001; Figure 1A) and CD8+ (17.0 vs 18.5; p=0.04; Figure 1B) T cells from PBMC. Similarly, in subcutaneous abdominal tissue mtDNA expression was lower among individuals with T2DM compared with individuals without T2DM in CD4+ (16.1 vs 19.0; p<0.001; Figure 1C) and CD8+ (16.0 vs 18.4; p<0.001; Figure 1D) T cells. Average mtDNA expression in CD8 and CD4 T cells was highly correlated across all individuals in PBMCs (R2=0.86, p<8.8E-4) and in adipose tissue (R2=0.66, p=0.014).

**Conclusion:** PLWH who have T2DM have reduced single cell mtDNA gene expression compared to those without T2DM in both circulating and subcutaneous adipose tissue CD4+ and CD8+ T cells. We used scRNA-seq to show an important link between mtDNA expression and T2DM. Future studies with longitudinal follow-up and larger sample sizes are needed to confirm these findings.

**Figure 1.** Normalized mtDNA expression plotted for each cell by individual. The average normalized mtDNA expression was compared between non-diabetic and diabetic individuals for peripheral blood mononuclear cells (PBMC) CD4+ T cells (A) PBMC CD8+ T cells (B) adipose tissue CD4+ T cells (C) and adipose tissue CD8+ T cells (D).

**523 CD4+ T EFFECTOR MEMORY CD45RA+ CELLS ARE ASSOCIATED WITH DIABETES IN PERSONS WITH HIV**

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**Background:** Changes in the distribution of T cell subsets have been linked to prevalent diabetes in the general population. As persistent immune activation is a hallmark of HIV infection, we assessed whether T cell subsets were associated with incident diabetes in persons with HIV (PWH).

**Methods:** We performed flow cytometry and functional assays on peripheral blood mononuclear cells collected from PWH and HIV-negative participants in the Veterans Aging Cohort Study between 2005 and 2007 to characterize CD4+ and CD8+ memory (central, effector, and effector RA+ [TEMRA]), CD57+, CD28-, and TH1, TH2, and TH17 CD4+ T cells. We used two definitions of TEMRA cells: CD45RA+CD28-CD57+ (TEMRA1) and CD45RA+CD27- (TEMRA2) (Table 1). Cases of incident diabetes were identified by two-physician chart adjudication. Individuals were followed until the onset of diabetes, death or end of study. We assessed PWH and HIV-negative participants separately using multivariable Cox proportional hazards models with T cell subset as the main exposure adjusted for demographic variables, lipid levels, cytomegalovirus (CMV) and hepatitis C virus serostatus, alcohol use, circulating inflammatory markers, and viral load and antiretroviral therapy use (PWH only). We report the hazard ratio (HR) for incident diabetes per standard deviation increment in the T cell subset.

**Cases:** The total study population included 2019 veterans (65% with HIV). In the HIV cohort, 105 (5%) of veterans had prevalent diabetes in the general population. As persistent immune activation is a hallmark of HIV infection, we assessed whether T cell subsets were associated with incident diabetes in persons with HIV (PWH).

**Methods:** We performed flow cytometry and functional assays on peripheral blood mononuclear cells collected from PWH and HIV-negative participants in the Veterans Aging Cohort Study between 2005 and 2007 to characterize CD4+ and CD8+ memory (central, effector, and effector RA+ [TEMRA]), CD57+, CD28-, and TH1, TH2, and TH17 CD4+ T cells. We used two definitions of TEMRA cells: CD45RA+CD28-CD57+ (TEMRA1) and CD45RA+CD27- (TEMRA2) (Table 1). Cases of incident diabetes were identified by two-physician chart adjudication. Individuals were followed until the onset of diabetes, death or end of study. We assessed PWH and HIV-negative participants separately using multivariable Cox proportional hazards models with T cell subset as the main exposure adjusted for demographic variables, lipid levels, cytomegalovirus (CMV) and hepatitis C virus serostatus, alcohol use, circulating inflammatory markers, and viral load and antiretroviral therapy use (PWH only). We report the hazard ratio (HR) for incident diabetes per standard deviation increment in the T cell subset.

**Results:** A total of 1259 PWH and 578 HIV-negative individuals were without diabetes at baseline and there were 238 incident diabetes events (133 [10.6%] in PWH and 105 [18.2%] in HIV-negative) over a median follow-up time of 8.6 years. The median age was 52 years, 69% were black, 95% were male, and 65% of PWH were virologically suppressed. In the adjusted model, a higher baseline proportion of CD4+ TEMRA cells, using both definitions, was associated with increased risk of incident diabetes in PWH only (TEMRA1 HR 1.16 [1.00,1.34]; p=0.05 and TEMRA2 HR 1.20 [1.04,1.38]; p=0.01) (Table 1). Higher proportion of CD4+ CD28- T cells approached significance in the model (HR 1.16 [0.99,1.36]; p=0.06) in PWH only. A similar association was not observed for CD8+ TEMRA cells, and no T cell subsets were associated with risk of incident diabetes in HIV-negative individuals.
Conclusion: Higher baseline proportion of CD4+, but not CD8+, TEMRA cells was associated with an increased risk of incident diabetes in PWH. As diabetes is common in PWH, understanding the potential role of T cells may provide insight into prevention or therapeutic strategies.

<table>
<thead>
<tr>
<th>HIV-infected (N = 176)</th>
<th>Persons with HIV (N = 1290)</th>
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<tbody>
<tr>
<td><strong>HIV positive (%)</strong></td>
<td><strong>HIV positive (%)</strong></td>
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<tr>
<td>CD4 T cell subset</td>
<td>Hazard Ratio per 30</td>
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<td>increment (95% CI)</td>
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<tr>
<td>CD4+CD45RA/CD28</td>
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<tr>
<td>CD4+CD45RA/CD27</td>
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</tr>
<tr>
<td>CD4+</td>
<td>1.0 (0.98-1.02)</td>
</tr>
</tbody>
</table>

Table 1. Multivariate Cox proportional hazards models, stratified by HIV status, assessing the relationship of baseline T cell subsets and incident diabetes adjusted for age, race, cytomegalovirus serostatus, viral load and antiretroviral therapy use (persons with HIV only), high-density lipoprotein, low-density lipoprotein, total cholesterol, time updated body mass index, hepatitis C virus serostatus, history of alcohol abuse, and circulating concentrations of interleukin-8, D-dimer, and soluble CD40L. CI, confidence interval; SD, standard deviation; Temo, T effector memoryRA.

524 CONVERGENCE OF INFECTIOUS AND NONCOMMUNICABLE DISEASES IN RURAL SOUTH AFRICA

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Background: There has been remarkable progress in the treatment of HIV throughout sub-Saharan Africa but data are limited on the prevalence and overlap of other significant causes of disease in HIV-endemic populations.

Methods: In a rural district of South Africa, we leveraged demographic and health surveillance infrastructure to estimate the population prevalence and geospatial distribution of HIV, active and lifetime tuberculosis, elevated blood glucose, elevated blood pressure and combinations of these. Adolescent and adult residents were offered multi-disease screening at mobile health camps near their homes. The health screening included WHO-STEP protocols for anthropomorphic and blood pressure measurements, HIV Ag-Ab enzyme immunoassay, HIV viral load and CD4 count, glycosylated hemoglobin (HbA1c) and TB screening (digital chest x-ray with automated image interpretation (CAD4TB), sputum Xpert MTB/RIF Ultra and liquid mycobacterial culture).

Results: 17,118 adolescents and adults were assessed. Overall, 52.1% (95% CI 51.3-52.9) had at least one active disease: 34.2% (95% CI 33.5-34.9) had HIV, 1.4% (95% CI 1.2-1.6) had active tuberculosis, 21.8% (95% CI 21.2-22.4) had lifetime tuberculosis, 8.5% (95% CI 8.1-8.9) had elevated blood glucose and 23.0% (95% CI 22.4-23.6) had elevated blood pressure. Appropriate treatment and control of disease was highest for HIV (76.3%), and lower for elevated blood pressure (40.0%), active tuberculosis (31.3%) and elevated blood glucose (63.9%). Disease prevalence differed significantly by sex, across age groups and geospatially: men had higher prevalence of active and lifetime tuberculosis, while women had extremely high prevalence of HIV in middle age and increasing prevalence of multiple and poorly controlled non-communicable diseases after the age of 50 years.

Conclusion: We found a convergence of infectious and non-communicable disease epidemics in a rural South African population, with HIV relatively well treated, but tuberculosis, elevated blood glucose and elevated blood pressure poorly diagnosed and treated. A public health response that expands the successes of HIV testing and treatment programme to provide multi-disease differentiated care targeted to specific populations is required to optimize health in such settings in sub-Saharan Africa.

525 COMORBIDITY BURDEN IN PEOPLE LIVING WITH HIV IN THE UNITED STATES

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Background: Antiretroviral (ARV) therapy is required life-long among people living with HIV (PLWH). It is important to understand the burden of comorbidities and comediations in PLWH to optimize clinical care.

Methods: Retrospective analyses of administrative claims data for commercial and Medicare Advantage enrollees from the Optum Research Database was conducted. PLWH cohort included adults (≥18 years) with ≥1 pharmacy claim for an ARV drug or HIV/AIDS diagnosis code in medical claims in 2018 calendar year (index date: earliest of pharmacy or HIV diagnosis claim date). Comparison cohort included adults without HIV (PLWoH), matched 2:1 with PLWH on age, gender, race, region and insurance type. Continuous health plan enrollment of 12 months (baseline) prior to, and 30 days (follow-up) after index date was required. Comorbidities were identified during baseline using ICD-9/10 diagnosis codes from medical claims. Charlson Comorbidity Index (CCI) was computed excluding HIV/AIDS. Baseline comediations were identified from pharmacy/medical claims using National Drug Codes. Differences in PLWH vs PLWoH accounted for the matched design and used a Z-test with robust errors for continuous variables and Rao-Scott test for categorical variables.

Results: A total of 20,256 PLWH and 40,512 matched PLWoH were analyzed. The mean age was 52 years, 80% were male, 46% were White, and 59% resided in South. 17,694 (87%) PLWH received ARV treatment (NRTI=94%; INSTI=63%; NNRTI=29%; PI=17%). Hypertension was the most common comorbidity followed by dyslipidemia and neuro psychiatric conditions in both PLWH and PLWoH. Presence of ≥3 comorbidities (Figure 1), and mean CCI were higher in PLWH than PLWoH (0.93 vs 0.61, p<0.001), respectively. Most comorbid conditions were more prevalent among PLWH compared to PLWoH except obesity, type 2 diabetes mellitus, and autoimmune disorders which were more common in PLWoH (all p<0.01). Polypharmacy (≥2 medications) was more prevalent among PLWH vs PLWoH (84% vs 61%, p<0.001). The most prevalent non-ARV comediations in PLWH vs PLWoH were cardiovascular medications, 47% vs 42%; antidepressants, 27% vs 18%; and chronic antibiotics, 15% vs 7%; respectively (all p<0.001).

Conclusion: Multimorbidity and polypharmacy were more prevalent in PLWH compared to matched PLWoH. The study findings suggest the need for clinicians to consider comorbidities and comediations when selecting ARV regimens to minimize drug interactions and adverse events and thereby improve patient outcomes.
HIV differentially impacts age-related comorbidity burden among US women and men

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Background: Age-related non-AIDS comorbidities (NACM) occur earlier and more frequently among people with HIV (PWH) than HIV-negative (HIV-) peers. HIV may also differentially impact the burden of NACM experienced by women vs men.

Methods: PWH and HIV- participants followed in the MACS/WIHS Combined Cohort Study (MWCCS) since 2008/2009 (when >80% of male/female participants used antiretroviral therapy) were included with outcomes measured up to 03/2019. Age, covariates, NACM prevalence, and NACM burden (total number out of 10) were summarized as of last observation. Unadjusted and adjusted (race, body mass index [BMI], smoking, drinking, crack/cocaine, socioeconomic status) linear regression models assessed the effects of HIV serostatus, age and sex on NACM burden.

Results: Women (2316 PWH, 922 HIV-) vs men (1452 PWH, 1239 HIV-) had higher in women vs men, particularly among PWH, and the distribution psychiatric illness, dyslipidemia, liver, and bone disease. NACM burden was higher in women vs men, particularly among PWH, and the distribution of specific NACM prevalence differed by sex. Given HIV is associated with differential effects on age-related comorbidities by sex, HIV serostatus- and sex-specific strategies for NACM screening and prevention are needed.

Effects of switch from 3DR to 2DR on inflammatory biomarkers

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Background: Because inflammation has been linked to HIV transcription in lymphoid tissues during ART-mediated viral suppression (VS), it is necessary to address the long-term effects of changing triple therapy (3DR) to 2-drug regimens (2DR) on plasma inflammatory markers.

Methods: Nested study in the Spanish AIDS Research Network (CoRIS). We selected HIV-infected ART-naive patients initiating 3DR from 2004 to 2017 who achieved VS in the first 48 weeks of ART and either remained on 3DR during their entire follow-up or were switched to 2DR (3TC+bPI; 3TC+DTG; DTG+RPV) at least 48 weeks of suppressive ART. 180 subjects were selected based on plasma availability and longer follow-up. We assessed the trajectories of inflammation markers (IL-6, hsCRP, macrophage activation (sCD163), monocyte activation (sCD14), coagulation (D-dimer) and markers of intestinal damage (IFABP) during VS using multivariate piecewise mixed models.

Results: We analyzed 619 plasma samples from 148 subjects (3DR, N=90; 2DR, N=58), mean age 38 (SD 10) years, 87% men, 67% MSM, mean CD4 nadir 278 (SD 185) cells/μL, median duration of VS 4.3 (3.6-6.2) years. Median time from ART initiation to censoring was 4.6 (3.2-6.2) years. Median time from VS to 2DR was 3.4 (1.8-5.2) years. Subjects with 3DR experienced a slow decline of IL-6, CRP, sCD14, sCD163 and D-dimers over time (figure A). In contrast, compared to 3DR, switching to 2DR was associated with increases in IL-6 (p=0.01), CRP (p=0.003) and D-dimer (p=0.001) after year 3 from VS, after adjusting for covariates. Compared to 3DR, 2DR was associated with higher risk of CRP quartile increase (aOR 3.3, 95%CI 1.1-10) and D-dimer quartile increase (aOR 3.2, 95%CI 1.1-13). The adjusted biomarker trajectories did not reveal a distinct pattern according to the type of 2DR used. We also studied cross-correlations among the biomarkers, and found sCD14 and sCD163 to be more highly correlated (figure B, Rho 0.438, p<0.0001).

Conclusion: In this observational study in virally suppressed individuals, maintaining 3DR was associated with a more favourable long-term anti-inflammatory profile than switching to 2DR. The potential clinical implications of these findings on the development of non-AIDS events deserve further investigation.
528 SOLUBLE IMMUNE COSTIMULATORY MOLECULES ARE PREDICTIVE OF NON-AIDS EVENTS

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**Background:** Despite suppressive antiretroviral therapy (ART), people with HIV (PWH) experience an increased risk of morbidity and mortality, in part due to chronic inflammation and immune dysfunction. Immune co-stimulatory molecules exist in soluble forms at normal physiological conditions and many are elevated in cancer, HIV infection, and other inflammatory diseases, suggesting they could serve as promising early predictive biomarkers of adverse outcomes in PWH. We aimed to identify relationships between plasma levels of soluble immune co-stimulatory molecules with the incidence of non-AIDS events (NAEs) utilizing a nested case-control study from the AIDS Clinical Trials Group ALLRT cohort.

**Methods:** Study participants were evaluated at baseline (pre-ART; 66 cases, 97 controls), 1 year post-ART (112 cases, 211 controls), and immediately preceding an event (89 cases, 163 controls). NAEs (cases) include myocardial infarction/MI, stroke, malignancy, serious bacterial infection, and non-acidental death. Matched controls had an event-free follow-up equal or greater than that of the relevant case. All participants were virally suppressed on ART at year 1 and malignancy (OR=1.8) pre-event. Gal-9 also correlated with markers associated with specific NAEs, MI/stroke (OR= 1.9) and death (OR=2.8) at year 1.9), p=0.04 and OR=1.6 (1.0, 2.3), p=0.03. Association at year 1 remained significant with adjustment for CD4 count. Higher levels of Gal-9 were associated with specific NAEs, MI/stroke (OR=1.9) and death (OR=2.1) at year 1 and malignancy (OR=1.8) pre-event. Gal-9 also correlated with markers previously assessed to be predictive of NAEs, including sTNFR-I, sTNFR-II, and suPAR, at each timepoint (all r≥0.45, p<0.0001).

**Conclusion:** Higher levels of CD27 were associated with increased risk of NAEs at each time point: baseline (unadjusted odds ratio (OR) per 1 IQR =2.1, p=0.008), year 1 (OR=1.6, p=0.001), pre-event (OR=2.1, p<0.001). Higher levels CD40 was associated with increased risk of NAEs at baseline (OR=1.8, p=0.019) and pre-event (OR=1.7, p=0.008). These associations remained after adjustments for HIV RNA levels and CD4 counts. Furthermore, examining specific NAEs, higher CD27 was associated with both increased risk of death and MI/stroke at multiple time points (OR=2.9-5.3) and CD40 associated with malignancy at baseline and pre-event (OR=2.3-4).

**Results:** Higher levels of CD27 were associated with increased risk of NAEs at each time point: baseline (unadjusted odds ratio (OR) per 1 IQR =2.1, p=0.008), year 1 (OR=1.6, p=0.001), pre-event (OR=2.1, p<0.001). Higher levels CD40 was associated with increased risk of NAEs at baseline (OR=1.8, p=0.019) and pre-event (OR=1.7, p=0.008). These associations remained after adjustments for HIV RNA levels and CD4 counts. Furthermore, examining specific NAEs, higher CD27 was associated with both increased risk of death and MI/stroke at multiple time points (OR=2.9-5.3) and CD40 associated with malignancy at baseline and pre-event (OR=2.3-4).

**Conclusion:** Soluble CD27 and CD40 are predictive of NAEs and may inform interventional studies aimed to reduce morbidity and mortality in PWH on suppressive ART.

529 PLASMA GALECTIN-9 AS A PREDICTOR OF NON-AIDS EVENTS DURING SUPPRESSIVE ART

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**Background:** People with HIV (PWH) on antiretroviral therapy (ART) still experience an increased risk of morbidity and mortality, which is partly driven by chronic inflammation. We previously demonstrated that soluble galectin-9 (Gal-9), a pleiotropic glycan-binding immunomodulatory protein, is elevated in PWH on ART and associated with markers of HIV persistence, neurological complications and indices of morbidity and mortality in HIV infection. Here, we aimed to identify relationships between Gal-9 and the occurrence of non-AIDS events (NAEs) in PWH on suppressive ART, utilizing a nested case-control study from the AIDS Clinical Trials Group ALLRT cohort.

**Methods:** Study participants were evaluated at baseline (pre-ART; 66 cases, 97 controls), 1 year post-ART (112 cases, 211 controls), and immediately preceding an event (89 cases, 162 controls). NAEs (cases) include myocardial infarction/myocardial infarction/MI, stroke, malignancy, serious bacterial infection, and non-acidental death. Matched controls had an event-free follow-up equal or greater than that of the relevant case. All participants were virally suppressed on ART at year 1 and matched for age (within 10 years, median 45 years), sex (84% male), pre-ART CD4+ T cell count (within 50 cells/mm³, median 213 cells/mm³), ART regimen at 1 year, and parent study. Soluble co-stimulatory molecules CD27, CD28, CD40, CD4+ T cell count (within 50 cells/mm³, median 213 cells/mm³), ART regimen at 1 year, and parent study. Soluble co-stimulatory molecules CD27, CD28, CD40, GTR, GTRL, HYEM, BTLA, and ICOS were measured by Luminesc. Conditional logistic regression analysis assessed associations of co-stimulatory molecules and events, adjusting for pertinent covariates at each timepoint; noteworthy associations used a threshold of an effect size (OR per one IQR) ≥1.5.

**Results:** Higher levels of CD27 were associated with increased risk of NAEs at each time point: baseline (unadjusted odds ratio (OR) per 1 IQR =2.1, p=0.008), year 1 (OR=1.6, p=0.001), pre-event (OR=2.1, p<0.001). Higher levels CD40 was associated with increased risk of NAEs at baseline (OR=1.8, p=0.019) and pre-event (OR=1.7, p=0.008). These associations remained after adjustments for HIV RNA levels and CD4 counts. Furthermore, examining specific NAEs, higher CD27 associated with both increased risk of death and MI/stroke at multiple time points (OR=2.9-5.3) and CD40 associated with malignancy at baseline and pre-event (OR=2.3-4).

**Conclusion:** Soluble CD27 and CD40 are predictive of NAEs and may inform interventional studies aimed to reduce morbidity and mortality in PWH on suppressive ART.

**Table 1:** Unadjusted and Adjusted Conditional Logistic Regression Models for Associations between Co-stimulatory Molecules and non-AIDS events at Three Time-points.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Baseline (pre-ART)</th>
<th>Year 1 (post-ART)</th>
<th>Pre-Event</th>
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<tbody>
<tr>
<td></td>
<td>N=163</td>
<td>N=203</td>
<td>N=252</td>
</tr>
<tr>
<td>Unadjusted OR (95% CI) per one IQR (p-value)</td>
<td>Range of Adjusted OR per one IQR, p-value</td>
<td>Range of Adjusted OR per one IQR, p-value</td>
<td>Range of Adjusted OR per one IQR, p-value</td>
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<td>CD27</td>
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<tr>
<td>CD28</td>
<td>1.0 (0.2, 3.1), p=0.32</td>
<td>1.1–1.3</td>
<td>1.6 (0.7, 3.3), p=0.001</td>
</tr>
<tr>
<td>CD40</td>
<td>1.6 (1.0, 2.9), p=0.02</td>
<td>1.5–1.8</td>
<td>1.3 (0.8, 2.1), p=0.11</td>
</tr>
<tr>
<td>GTR</td>
<td>1.2 (0.8, 1.8), p=0.37</td>
<td>1.2–1.3</td>
<td>1.5 (0.7, 1.2), p=0.01</td>
</tr>
<tr>
<td>GTRL</td>
<td>1.4 (0.2, 4.1), p=0.18</td>
<td>1.3–1.4</td>
<td>1.4 (0.8, 2.1), p=0.18</td>
</tr>
<tr>
<td>HYEM</td>
<td>1.5 (0.2, 2.0), p=0.09</td>
<td>1.0–1.3</td>
<td>1.2 (0.1, 2.3), p=0.09</td>
</tr>
<tr>
<td>BTLA</td>
<td>1.6 (0.2, 3.1), p=0.23</td>
<td>1.2–1.4</td>
<td>1.6 (0.7, 3.3), p=0.05</td>
</tr>
<tr>
<td>ICOS</td>
<td>1.4 (0.2, 2.2), p=0.19</td>
<td>1.3–1.4</td>
<td>1.5 (0.7, 3.3), p=0.05</td>
</tr>
</tbody>
</table>

**Abbreviations:** OR, odds ratio; CI, confidence interval; IQR, interquartile range; p-value

**Adaptations were done individually for the following covariates:** 1) HIV disease stage (Baseline: log10 HIV RNA level, Pre-ART: CD4+ T cell count). 2) Time updated anti-retroviral therapy status. 3) Time updated smoking status. 4) Baseline second-line drug use. 5) Time updated weight to BMI ratio. 6) Time updated diabetes status. 7) Time updated hypertension status. 8) Time updated use of antidepressants or lipid lowering medications. 9) Time updated family history of myocardial infarction.
FEMINIZING THERAPY DECREASES D-DIMER BUT WORSENS INSULIN SENSITIVITY IN TRANS WOMEN

Jordan Lake, Hongyu Miao, Aaren Kettelhut, Jesse Clark, Javier R. Lama, Sari Reisner, Kenneth H. Mayer, Amaya Perez-Brumer, Nicholas Funderburg

UTHealth, Houston, TX, USA, O&M School of Medicine, Santo Domingo, Dominican Republic, University of California Los Angeles, Los Angeles, CA, USA, Asociación Civil Impacta Salud y Educación, Lima, Peru, Brigham and Women’s Hospital, Boston, MA, USA, Fenway Health, Boston, MA, USA, University of Toronto, Toronto, Canada, Ohio State University, Columbus, OH, USA

Background: Feminizing hormonal therapies (FHT) and HIV are known to increase cardiovascular risk for transgender women (TW), though little data exists to quantify cardiometabolic changes following FHT initiation, particularly among TW living with HIV (HIV+).

Methods: The Féminas study enrolled 220 TW from October 2016-March 2017 in Lima, Peru. Participants were of HIV negative (HIV-) or unknown serostatus. All received HIV/STI testing and access to FHT (estradiol valerate (i.e., condomless intercourse, partner discordance) for HIV acquisition and/or transmission. All received HIV/STI testing and access to FHT (estradiol valerate and spironolactone), PrEP (TDF/FTC) or ART (TDF/FTC/EFV) for 12 months. Serum was stored for cardiometabolic biomarker measurement; fasting glucose and triglycerides were measured in real time.

Results: 171 TW (32 HIV-, 139 HIV+) had stored samples for analysis. Median age was 26 years, 70% had history of prior FHT use. At baseline, PCSK9, sCD14, TNFα, CRP and EN-RAGE levels were significantly (p<0.05) higher in HIV+ vs HIV- TW, whereas HDL and total cholesterol were lower, and insulin and glucose parameters were similar (data not shown). All HIV+ TW started ART, but only 5 achieved HIV RNA <50 copies/mL at any time on ART adherence was very low, these effects seem primarily due to FHT use.

Conclusion: In this unique cohort, FHT initiation appeared to decrease d dimer but worsen insulin sensitivity for both HIV+ and HIV- TW. Because PrEP uptake and ART adherence were very low, these effects seem primarily due to FHT use. Further study is needed to better understand cardiometabolic changes in TW by HIV serostatus and to optimize uptake of, and adherence to, HIV prevention and treatment options.
532  INDEPENDENT ASSOCIATIONS OF TNF-ALPHA AND IL-1 BETA WITH EMPHYSEMA IN HIV INFECTION


HighHospitalet, Copenhagen, Denmark, *Hvidøre Hospital, Hvidøre, Denmark, Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CUREME), University College London, London, UK, Oslo University Hospital, Oslo, Norway, Statens Serum Institut, Copenhagen, Denmark, University of Manchester, Manchester, UK.

**Background:** Emphysema has been suggested to occur more frequently and at younger age in people living with HIV (PLWH) than in uninfected controls. Risk factors for emphysema include smoking and alpha-1 antitrypsin (AAT) deficiency, but recent evidence suggest that inflammation may also play a role. We investigated whether elevated cytokine concentrations (interleukin(IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-6, IL-10, IL-17A, tumor necrosis factor-alpha (TNFα), interferon-gamma (IFNγ), soluble CD14 (sCD14) and sCD163) are independently associated with radiographic emphysema in PLWH.

**Methods:** We included PLWH without hepatitis B and C co-infection with a plasma sample and a chest computed tomography scan available from the Copenhagen Comorbidity in HIV Infection (COCOMO) Study. Radiographic emphysema plus trace emphysema was defined as percentage of low attenuation area under 950 Hounsfield units (%LAA-950) using a cut-off at 5%. Cytokine concentrations were measured by ELISA or Luminex immunoassays, and an elevated cytokine concentration was defined as above the 75th percentile. Logistic regression analyses were performed to explore associations between elevated cytokine concentrations and radiographic emphysema plus trace emphysema in PLWH.

**Results:** Of 783 PLWH, 147 (18.8%) had emphysema plus trace emphysema. PLWH were predominantly male (86.0%) and 743 (94.9%) had undetectable viral load. PLWH with emphysema plus trace emphysema had higher concentrations of TNFα (median (IQR): 8.2 (6.4–9.8) versus 7.1 (5.7–8.6) pg/ml, p<0.001), IL-1β (median (IQR): 0.21 (0.1–0.4) versus 0.17 (0.1–0.3) pg/ml, p=0.004) and IL-6 (median (IQR): 3.6 (2.6–4.9) versus 3.1 (2.0–4.3) pg/ml, p=0.023) than PLWH without. AAT deficiency (<1.0 g/L) was rare and equally prevalent in the two groups (p=0.291). In models adjusted for age, sex, ethnicity, smoking status, BMI, and CD4 nadir, elevated TNFα (adjusted odds ratio (aOR): 1.91 [95%CI: 1.23–2.95], p=0.004) and IL-1β (aOR: 1.93 [95%CI: 1.25–2.99], p=0.004) were associated with emphysema plus trace emphysema (Table 1).

**Conclusion:** Two markers of systemic inflammation, TNFα and IL-1β, were independently associated with radiographic emphysema plus trace emphysema in PLWH. Of 783 PLWH, 147 (18.8%) had emphysema plus trace emphysema. PLWH were predominantly male (86.0%) and 743 (94.9%) had undetectable viral load. PLWH with emphysema plus trace emphysema had higher concentrations of TNFα (median (IQR): 8.2 (6.4–9.8) versus 7.1 (5.7–8.6) pg/ml, p<0.001), IL-1β (median (IQR): 0.21 (0.1–0.4) versus 0.17 (0.1–0.3) pg/ml, p=0.004) and IL-6 (median (IQR): 3.6 (2.6–4.9) versus 3.1 (2.0–4.3) pg/ml, p=0.023) than PLWH without. AAT deficiency (<1.0 g/L) was rare and equally prevalent in the two groups (p=0.291). In models adjusted for age, sex, ethnicity, smoking status, BMI, and CD4 nadir, elevated TNFα (adjusted odds ratio (aOR): 1.91 [95%CI: 1.23–2.95], p=0.004) and IL-1β (aOR: 1.93 [95%CI: 1.25–2.99], p=0.004) were associated with emphysema plus trace emphysema (Table 1).

**Conclusion:** Two markers of systemic inflammation, TNFα and IL-1β, were independently associated with radiographic emphysema plus trace emphysema in PLWH. Of 783 PLWH, 147 (18.8%) had emphysema plus trace emphysema. PLWH were predominantly male (86.0%) and 743 (94.9%) had undetectable viral load. PLWH with emphysema plus trace emphysema had higher concentrations of TNFα (median (IQR): 8.2 (6.4–9.8) versus 7.1 (5.7–8.6) pg/ml, p<0.001), IL-1β (median (IQR): 0.21 (0.1–0.4) versus 0.17 (0.1–0.3) pg/ml, p=0.004) and IL-6 (median (IQR): 3.6 (2.6–4.9) versus 3.1 (2.0–4.3) pg/ml, p=0.023) than PLWH without. AAT deficiency (<1.0 g/L) was rare and equally prevalent in the two groups (p=0.291). In models adjusted for age, sex, ethnicity, smoking status, BMI, and CD4 nadir, elevated TNFα (adjusted odds ratio (aOR): 1.91 [95%CI: 1.23–2.95], p=0.004) and IL-1β (aOR: 1.93 [95%CI: 1.25–2.99], p=0.004) were associated with emphysema plus trace emphysema (Table 1).

**Table 1:** Multivariable logistic regression analyses for the association between elevated cytokine concentration (above the 75th percentile) and emphysema plus trace emphysema in PLWH.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>1.91</td>
<td>[1.23–2.95]</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.93</td>
<td>[1.25–2.99]</td>
</tr>
</tbody>
</table>

**533  ASSOCIATION OF NADIR CD4/CD8 WITH CVD AND NON-AIDS–DEFINING CANCERS IN THE DC COHORT**

Letumile R. Moeng, Morgan Byrne, Anne Monroe, Vishnu Priya Mallipelli, Michael A. Horberg, Amanda Castle, Ronald Wilcox, for the DC Cohort Executive Committee.

Howard University, Washington, DC, USA, *George Washington University, Washington, DC, USA, Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA.

**Background:** HIV has transitioned from a progressive, fatal disease to a chronic, manageable disease marked by elevated risk of cardiovascular diseases (CVD). Rates of myocardial infarction, heart failure, stroke and other CVD manifestations are significantly higher for people with HIV (PWH), even with antiretroviral therapy (ART). The era of ART has also seen an increase in the burden of non-AIDS defining cancers (NADC). Low CD4/CD8 has been associated with increased risk of CVD and NADC among PWH in previous studies. Data on clinical use of nadir CD4/CD8 is limited. To address this knowledge gap, we investigated the association between nadir CD4/CD8 and CVD and NADC.

**Methods:** We investigated the association between nadir CD4/CD8 and CVD and NADC. A nadir CD4/CD8 <0.5 was associated with increased odds of CVD (OR 1.94; 95% CI 1.6–2.3) and NADC (OR 1.94; 95% CI 1.6–2.2). In a large urban cohort of PLWH, a nadir CD4/CD8 <0.5 was associated with increased CVD and NADC. Low nadir CD4/CD8 likely portends increased risk and could be a useful screening measure for CVDs and NADC among PWH.

**Table 1:** Multivariable logistic regression for Cardiovascular and Non-AIDS associated Cancer controlling for Demographic, Clinical, and predictors of disease.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CVD</th>
<th>NADC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at CD4 nadir, y</td>
<td>&lt;0.6 (0.4–1.0)</td>
<td>&lt;0.6 (0.4–1.0)</td>
</tr>
<tr>
<td>Gender</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Male</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Female</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>Non-Hispanic Black</td>
<td>Non-Hispanic White</td>
</tr>
<tr>
<td>Black</td>
<td>1.06 (0.7–1.5)</td>
<td>1.06 (0.7–1.5)</td>
</tr>
<tr>
<td>White</td>
<td>0.90 (0.5–1.6)</td>
<td>0.90 (0.6–1.5)</td>
</tr>
<tr>
<td>Transgender</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>1.27 (0.9–1.8)</td>
<td>1.27 (0.9–1.8)</td>
</tr>
<tr>
<td>White</td>
<td>0.80 (0.5–1.3)</td>
<td>0.80 (0.5–1.3)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>2.22 (1.1–4.2)</td>
<td>2.22 (1.1–4.2)</td>
</tr>
<tr>
<td>Prevalent smoker</td>
<td>1.13 (0.6–2.0)</td>
<td>1.13 (0.6–2.0)</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>1.05 (0.6–1.6)</td>
<td>1.05 (0.6–1.6)</td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>0.5 (0.3–0.9)</td>
<td>0.5 (0.3–0.9)</td>
</tr>
<tr>
<td>Obesity</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
<tr>
<td>CVD</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
<tr>
<td>NADC</td>
<td>0.80 (0.4–1.6)</td>
<td>0.80 (0.4–1.6)</td>
</tr>
</tbody>
</table>

*534 Participants included in multivariable logistic regression model
*535 Participants included in multivariable logistic regression model
534 EPGENETIC AGING CHANGES IN A COHORT OF AVIREMIC HIV-INFECTED ADULTS
Andrés Esteban-Cantos1, Javier Rodríguez-Centeno1, Gabriel Saiz-Medrano1, Rocío Montejano1, Rosa De Miguel1, Pilar Baruz1, Julián Nevada1, Beatriz Mena-Garay1, Jose I. Bernardino1, Julen Cadiñanos1, Fabiola Valenza1, Rafael Mican1, Mario Mayoral-Muñoz1, Jose R. Arribas1, Berta Rodes1, Berta Rodes1, Cadiñanos1, Stella-Ascariz1, La Paz University Hospital, Madrid, Spain

Background: Untreated HIV infection causes accentuated epigenetic aging which is partially reversed by antiretroviral therapy (ART) initiation (Lancet HIV, in press). It remains unclear whether epigenetic aging continues to accelerate over time in people living with HIV (PLWH) receiving suppressive ART.

Methods: We analyzed 63 participants from a prospective cohort of long-term treated aviremic HIV-infected adults at two timepoints (baseline and 4 years of follow-up). Whole blood DNA methylation profiles were obtained by Illumina Infinium MethylationEPIC BeadChip. We evaluated 4 epigenetic clocks (Horvath and Hannum’s clocks, PhenoAge and GrimAge), and calculated epigenetic age acceleration (EAA) for each one as the residuals resulting from the regression of epigenetic age on chronological age using a mixed-effects linear regression model.

Results: At baseline, participants were predominantly male (82.5%) and Caucasian (93.7%), median chronological age was 48.7 years (IQR 44.2-53.5), median time since HIV infection diagnosis was 18 years (IQR 13.7-22.3) and median CD4 cell counts was 770 cells/µl (IQR 575-922). 32 participants (50.8%) were receiving a tenofovir (TFV) containing regimen and 31 (49.2%) were never exposed to TFV (of whom 15 were receiving abacavir and 16 a nucleosid[e] reverse transcriptase inhibitor sparing regimen). All participants were male, 88% black, 52% overweight or obese, 6% had severe drug abuse, and 33% hazardous alcohol drinking at index visit. Compared to people without PWID, participants were younger (P=0.005), more likely to be African American (P=0.01), and have a significantly higher percentage of PWID (P=0.03). Compared to participants with PWID, participants were significantly more likely to be female (P=0.01), have lower CD4 + T-cells counts (P=0.01), and be younger (P=0.01). In long-term aviremic PLWH, TFV treatment was associated with shorter telomeres in CD8+ T-cells, possibly due to the inhibition of telomerase activity. Treatment with TFV was not associated with changes in T cells subpopulations distribution.

Conclusion: Epigenetic aging did not accelerate in a cohort of aviremic HIV-infected adults after four years of follow-up, independently of the antiretroviral regimen and CD4 changes. These results are not consistent with an accelerated biological aging process during successfully treated HIV infection.

535 EFFECT OF TENOFOVIR ON TELOMERASE, TELOMERE LENGTH, AND T-CELLS IN AVIREMIC HIV ADULTS
Javier Rodríguez-Centeno1, Andrés Esteban-Cantos1, Rocío Montejano1, Natalia Stella-Ascariz1, Beatriz Mena-Garay1, Maria Jimenez-Gonzalez1, Rosa De Miguel1, Berta Rodes1, Belen Alejos1, Gabriel Saiz-Medrano1, Jose I. Bernardino1, Julen Cadiñanos1, Juan M. Castro-Álvarez1, Jésus R. Arribas1, La Paz University Hospital, Madrid, Spain

Background: Tenofovir (TFV), at therapeutic concentrations, inhibits telomerase activity (TA) in vitro in activated PBMCs from healthy volunteers (J Acquir Immune Defic Syndr 2017; 74:91) and compared to non-TFV-containing regimens, TFV-containing treatments produced treatments produced smaller gains in whole blood telomere length (TL) after 2 years of follow-up in PLWH with prolonged virological suppression (J Infect Dis 2018; 218:1531). We now report ex vivo, effects of TFV on TL and TA, in PBMCs, CD4+ and CD8+ T-cells and on T-cell subsets distribution in long-term aviremic HIV+ persons.

Methods: We analyzed HIV participants with suppressed virological replication who have been continuously receiving TFV for at least 4 years (TDF, n=23 or TAF, n=27) or never exposed to TFV: Abacavir (n=60) and NRTI-sparing regimens (n=21). PBMCs were isolated using density gradient media. CD4+ and CD8+ T-cells were isolated from fresh PBMCs by magnetic separation. We measured TL (T5 ratio) with monochrome multiplex qPCR and TA using the TRApeze RT Telomere Detection Kit. CD4+ and CD8+ T-cell subsets were analyzed by flow cytometry from fresh PBMCs: Recent Thymic Emigrants (RTE) (CD31+CD45RA-CD27-), naive (CD45RA+CD27+), central Memory (CD45RA-CD27+), effector memory (CD45RA-CD27+), TEMRA (CD45RA+CD27-), senescent (CD28-CD57+), exhausted (PD1+) and activated (CD38+HLA-DR+).

Results: At baseline, participants were predominantly male (80.3%) and Caucasian (93.7%), median chronological age was 48.7 years (IQR 44.2-53.5), median time since HIV infection diagnosis was 18 years (IQR 13.7-22.3) and median CD4 cell counts was 770 cells/µl (IQR 575-922). 32 participants (50.8%) were receiving a tenofovir (TFV) containing regimen and 31 (49.2%) were never exposed to TFV (of whom 15 were receiving abacavir and 16 a nucleos[ide] reverse transcriptase inhibitor sparing regimen). All participants were male, 88% black, 52% overweight or obese, 6% had severe drug abuse, and 33% hazardous alcohol drinking at index visit. Compared to people without PWID, participants were significantly more likely to be female (P=0.01), have lower CD4 + T-cells counts (P=0.01), and be younger (P=0.01). In long-term aviremic PLWH, TFV treatment was associated with shorter telomeres in CD8+ T-cells, possibly due to the inhibition of telomerase activity. Treatment with TFV was not associated with changes in T cells subpopulations distribution.

Conclusion: In long-term aviremic PLWH, TFV treatment was associated with shorter telomeres in CD8+ T-cells, possibly due to the inhibition of telomerase activity. Treatment with TFV was not associated with changes in T cells subpopulations distribution.

536 SERUM LEVEL OF CELL-FREE DNA FRAGMENTS IS ASSOCIATED WITH FRAILTY AND SEVERITY OF HIV
Jing Sun1, Lolita S. Nidadavolu1, Jacque Astemborski2, Damani A. Piggott1, Thomas Laskow1, Druth H. Mehta1, Todd Brown1, Gregory D. Kink1, Peter Abadir1, for the AIDS Linked to the IntraVenous Experience (ALIVE)
1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: After cellular death, circulating cell-free mitochondrial (ccf-mtDNA) and genomic (ccf-gDNA) DNA fragments are released into circulation. The levels of ccf-gDNA fragments can reflect cell turnover, and ccf-mtDNA fragments can be used to distinguish the mode of cell death (apoptotic vs. necrotic) based on their size (short vs. long fragments, respectively). The utility of serum ccf-gDNA as a biomarker and indication of aging among people with HIV (PWHL) is unclear.

Methods: Using ultrasensitive digital PCR, ccf-gDNA and ccf-mtDNA were quantified in serum among HIV-infected and uninfected participants in the AIDS Linked to the IntraVenous Experience (ALIVE) cohort of current and former persons who inject drugs (PWID). Fried frailty phenotype was measured at semi-annual study visits. Linear regression models with generalized estimating equations (GEE) were used to compare differences in log-transformed circulating cell-free DNA fragments by HIV markers after adjusting for covariates (demographics, BMI, drug/alcohol abuse, and white blood cell count). The relationship of ccf-gDNA to frailty was assessed using multinomial logistic regression with GEE.

Results: At baseline, participants were predominantly male (80.3%) and Caucasian (93.7%), median chronological age was 48.7 years (IQR 44.2-53.5), median time since HIV infection diagnosis was 18 years (IQR 13.7-22.3) and median CD4 cell counts was 770 cells/µl (IQR 575-922). 32 participants (50.8%) were receiving a tenofovir (TFV) containing regimen and 31 (49.2%) were never exposed to TFV (of whom 15 were receiving abacavir and 16 a nucleos[ide] reverse transcriptase inhibitor sparing regimen). All participants were male, 88% black, 52% overweight or obese, 6% had severe drug abuse, and 33% hazardous alcohol drinking at index visit. Compared to people without PWID, participants were significantly more likely to be female (P=0.01), have lower CD4 + T-cells counts (P=0.01), and be younger (P=0.01). In long-term aviremic PLWH, TFV treatment was associated with shorter telomeres in CD8+ T-cells, possibly due to the inhibition of telomerase activity. Treatment with TFV was not associated with changes in T cells subpopulations distribution.
537 PREVALENCE AND DETERMINANTS OF FRAILTY IN PLHIV AGED 70+: ANRS SEPTAVIH STUDY

Clotilde Allavena1, Hubert Blain1, Xian Abulizi2, Mariem Raho Moussa3, Frank Palella4, Erin D. Michos5, Kathleen Weber6, Christine Katlama7, Charles Cazanave8, Hubert Blain9, Deborah Gustafson10, Laurence3, Deborah Jones Weiss11, Pascal Pugliese12, Carole Cagnot13, Elizabeth Vásquez14, Xian Abulizi1, Allison Appleton2, 7

EP66 SEPTAVIH multicenter prospective study aims to assess frailty in older PLHIV has been evaluated in middle-aged people living with HIV (PLHIV). The ANRS EP66 SEPTAVIH multicenter prospective study aims to assess frailty in older PLHIV on antiretroviral treatment.

Methods: In 2019, SEPTAVIH enrolled PLHIV aged 70 years or older on antiretroviral treatment for > 12 months from 16 centers in France. Sociodemographic, clinical data and medical/HIV history were collected at baseline. A comprehensive geriatric interview and examination assessed the history and risks of falls, associated medication, physical and cognitive function (MoCA), and mood disorders (CES-D). Frailty was assessed using the 5 criteria Fried frailty phenotype (FFP); recent spontaneous weight loss, low hand grip strength, exhaustion, slow walking speed, and low physical activity. PLHIV were classified as robust (no criteria), pre-frail (1 or 2 criteria) and frail (> 2 criteria). We compared, among frailty categories, continuous variables with Kruskal-Wallis tests and categorical variables using Chi-2 tests.

Results: 510 PLHIV were included. Subjects had a median age of 73 years (IQR:71-77); 31.3% were >75 years; 81.4% were male (of which 58.1% MSM), 80% were born in France, 27.4% had a history of AIDS, 40.3% had a college education level, and 62.6% were homemakers. At baseline, median HIV duration was 22.7 years, and PLHIV had a median of 3 comorbidities (IQR:2-4); median CD4 cell count was 562.0/µL [418-752] and plasma HIV-RNA< 50 copies/mL in 95.3%. Depression and cognitive impairment were present in 24.7% and 59.4%, respectively. 13.5% of the subjects were frail, 63.2% pre-frail, and 23.4% robust. Spontaneous weight loss was observed in 9.7%, low hand grip strength in 56.3%, exhaustion in 31.8%, slow walking speed in 21.5% and low physical activity in 14.8%. Older age, increased comorbidities, lower education level and socioeconomic status were associated with frailty (Table). Lower cognitive performance was more prevalent in pre-frail and frail subjects. Conversely, HIV-related parameters were not associated with frailty.

Conclusion: Prevalence of frailty was low in PLHIV aged 70 or older, though nearly two thirds were prefrail. Socio-economic conditions, comorbidities and cognitive function more than HIV-related factors were the main contributors of frailty, suggesting the need of targeted interventions in aging PLHIV with controlled HIV disease.

538 CARDIOVASCULAR RISK SCORE ASSOCIATIONS WITH FRAILTY IN WHIHS AND MACS

Mark H. Kuniholm1, Elizabeth Vásquez1, Allison Appleton2, Frank Palella2, Matthew Budoff3, Erin D. Michos4, Deborah Jones Weiss5, Adaora Adimora6, Igbo Ofoftokun7, Gyspysmber D’Souza8, Kathleen Weber9, Phyllis Tiffen4, Michael Plankey10, Anjali Sharma11, Deborah Gustafson12

1University at Albany, Albany, NY, USA, 2Northwestern University, Chicago, IL, USA, 3University of California Los Angeles, Los Angeles, CA, USA, 4Johns Hopkins University, Baltimore, MD, USA, 5University of Miami, Miami, FL, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 7Emory University, Atlanta, GA, USA, 8Cook County Health and Hospitals System, Chicago, IL, USA, 9University of California San Francisco, San Francisco, CA, USA, 10Georgetown University, Washington, DC, USA, 11Albert Einstein College of Medicine, Bronx, NY, USA, 12State University of New York Downstate Medical Center, Brooklyn, NY, USA

Background: The relationship between cardiovascular disease (CVD) and frailty among people living with HIV (PLWH) is not well established. We hypothesized that 10-year coronary heart disease and atherosclerotic CVD risk computed by Framingham risk score (FRS - 2001 National Cholesterol Education Program Adult Treatment Program III) and Pooled Cohort Equations (PCE - 2013 American College of Cardiology/American Heart Association guidelines) would correlate with the Fried Frailty Phenotype (FFP) in the Women's Interagency HIV Study (WIHS) and Multicenter AIDS Cohort Study (MACS). The primary objectives were to: 1) estimate associations of FRS and PCE risk with frailty in women and men over 3 time windows and 2) determine whether FRS and/or PCE risk predict incident frailty in men.

Methods: FFP was ascertained in the WIHS during 2004-2006 and 2011-2019, and in the MACS during 2007-2019. FFP score >3 defined frailty. Repeated measures logistic regression, log-rank and Cox proportional hazards regression were performed adjusted for education, income, cholesterol medication use, HIV serostatus, CD4 cell count<350, ART use, and HIV viral load.

Results: There were 5,554 participants (813 HIV-/2,025 HIV+ women; 1,303 HIV-/1,413 HIV+ men) who had FRS and FFP data at ≥ 1 visit and 3,630 (494 HIV-/1,413 HIV+ men) with PCE available. High risk FRS [adjusted odds ratio(aOR)=1.41(95% CI:1.11-1.80) and aOR=1.43(95% CI:1.20-1.70) but not high risk FRS (aOR=1.29(95% CI:0.46-3.30) and aOR=0.60(95% CI:0.17-2.15) was associated with higher odds of frailty in HIV+ women, respectively. In men, both high risk FRS (aOR=2.31(95% CI:1.74-3.07) and aOR=2.07(95% CI:1.65-2.60); HIV interaction PInt=0.63) and high risk PCE (aOR=2.12(95% CI:1.78-2.51) and aOR=1.43(95% CI:1.25-1.63); PInt=0.0008) were associated with higher odds of frailty in HIV+ men, respectively. In log-rank tests (Figure) and adjusted Cox models, high risk FRS (adjusted hazard ratio(aHR)=2.53(95% CI:4.94-3.2) and aHR=1.96(95% CI:1.6-3.3); PInt=0.95) and high risk PCE aHR=1.80(95% CI:3.9-2.3) and
Figure 1: Kaplan-Meier curves for frailty-free survival (A) AKI-1 group, (B) AKI-2 group, (C) AKI-3 group, and (D) controls. The log-rank test showed statistically significant differences among the groups (p<0.001).

539 BEYOND PAIN: PRESCRIPTION OPIOID USE IN THE WOMEN’S INTERAGENCY HIV STUDY

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Background: Women are more likely than men to experience chronic pain and use prescription opioids. Less is known about predictors and consequences of prescription opioid use among women living with HIV (WLWH).

Methods: 3,826 WLWH or women without HIV enrolled in the Women’s Interagency HIV Study (WHIS) and completed bi-annual self-report prescription opioid use assessments from 2000-2019. Cumulative proportion of visits with prescription opioid use was categorized as: minimal (0-9%); intermediate (10-39%); and chronic (40% or greater). Logistic regression examined independent predictors and proportional hazards regression estimated the unadjusted hazard of all-cause mortality, comparing intermediate and chronic prescription opioid use with those with minimal opioid use. Women with HIV RNA <200 c/mL and a history of PRT (confirmed by dual X-ray absorptiometry. Renal and bone biomarkers were analysed using multi-level mixed effects linear regression models. The trial was registered under EudraCT 2016-003345-29.

Results: 31 individuals were enrolled (mean age 50.1 [SD 16.6] years, 97% male, 13% black ethnicity, median [IQR] time since HIV diagnosis, first ART exposure, and stopping TDF: 19.4 [12.2, 26.2], 12.6 [7.5, 21.0], and 6.9 [5.4, 10.1] years, respectively, and median CD4 count 489 [429, 637] cells/mm³); all remained on F/TAF at week 96; none developed glycosuria or PRT; 13 simplified their HIV/HBV regimen were allowed. Bone mineral density (BMD) was measured annually by dual X-ray absorptiometry. Renal and bone biomarkers were analysed using multi-level mixed effects linear regression models. The trial was registered under EudraCT 2016-003345-29.

Results: At index visit, most participants were Black (64%), with mean age of 38.9 years and a median annual income of < $12,000. Women contributed 82,396 person-visits over a median of 10.6 (interquartile range [IQR] = 5.0-17.2) years. The annual prevalence of prescription opioid use significantly increased from 12.6% to 19.3% from 2000-2019 (p < 0.0001). Prescription opioid use was classified as minimal in 75%, intermediate in 16%, and chronic in 9% of women. WLWH had 56% higher odds of chronic prescription opioid use compared to women without HIV. Even after adjusting for quality of life scores that included ratings of pain, women with intermediate prescription opioid use had greater odds of being sexual minorities (lesbian or bisexual), unemployed, and were more likely to report benzodiazepine and non-prescription substance use compared to those with minimal use. Similar results were found when comparing chronic to minimal opioid use, but with generally greater magnitude than those observed with intermediate versus minimal use (see Table). Intermediate and chronic prescription opioid use were respectively associated with a 2-fold and 1-fold increased risk of all-cause mortality.

Conclusion: Despite federally mandated changes in opioid prescribing guidelines, prescription opioid use in the WHIS significantly increased from 2000-2019. Findings underscore the need for non-opioid and non-pharmacologic interventions for chronic pain in sexual minorities and WLWH. Concurrent use of benzodiazepines and non-prescription drugs should also be addressed and treated to reduce risk of mortality.

540 SAFETY OF TAF IN PATIENTS WITH A HISTORY OF PROXIMAL RENAL TUBULOPATHY ON TDF

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1King’s College Hospital NHS Foundation Trust, London, UK, 2St George’s University of London, London, UK, 3Imperial College London, London, UK, 4Brighton and Sussex University Hospitals NHS Trust, Brighton, UK, 5Chelsea and Westminster Hospital, London, UK, 6Royal Free Hospital, London, UK, 7UK Community Advisory Board, London, UK

Background: TDF may cause treatment-limiting proximal renal tubulopathy (PRT). We studied the safety of tenofenov alafenamide (TAF) in individuals who had developed PRT while receiving tenofen disoproxil fumarate (TDF) containing antiretroviral therapy (ART).

Methods: Individuals with HIV RNA <200 c/mL and a history of PRT (confirmed historically or by ≥2 of: proteinuria [PCR >30 mg/mmol], hypophosphatemia [phosphate <0.64 mmol/L], normoglycemic glycosuria, rapid eGFR decline (>5ml/min/1.73m²/year), clinical resolution upon TDF discontinuation, and not currently receiving TDF or TAF were initiated on entecavir/tenofen or TAF and followed quarterly for 96 weeks. Changes to other components of the ART regimen were allowed. Bone mineral density (BMD) was measured annually by dual X-ray absorptiometry. Renal and bone biomarkers were analysed using multi-level mixed effects linear regression models. The trial was registered under EudraCT 2016-003345-29.

Results: 31 individuals were enrolled (mean age 50.1 [SD 16.6] years, 97% male, 13% black ethnicity, median [IQR] time since HIV diagnosis, first ART exposure, and stopping TDF: 19.4 [12.2, 26.2], 12.6 [7.5, 21.0], and 6.9 [5.4, 10.1] years, respectively, and median CD4 count 489 [429, 637] cells/mm³); all remained on F/TAF at week 96; none developed glycosuria or PRT; 13 simplified their HIV/ HBV regimen. Changes in median values for renal and bone biomarkers are displayed in Fig 1. Participants experienced small declines in creatinine-based eGFR (-2.5 [95%CI -4.2, -0.8]), but not cystatin C-based eGFR (-0.9 [-2.1, 0.4] mL/min/1.73m²/year), and increases in RBPCR (6.3 [3.8, 10.7] µg/mmol/year) (p<0.0001); albuminuria, fractional excretion of phosphate, markers of bone turnover and BMD remained stable (p>0.1). Five participants (16%) had RBPCR >10xULN (two ongoing from baseline): none had confirmed hypophosphatemia, four had proteinuria (all ongoing and improved from baseline), none had rapid eGFR-cystatin C decline, all had stable FE-PO4 and all continued F/TAF post week 96.

Conclusion: In individuals with a history of PRT on TDF, 96-weeks of exposure to TAF was not associated with recurrent PRT. RBPCR, a marker of tubular dysfunction, increased over time. A subset of participants had substantial elevations of RBPCR although these were not accompanied by other markers of tubular dysfunction. Whilst these data are encouraging, follow up will continue for five years to confirm the long-term safety of F/TAF in this population.
Table 1. Data From ATLAS/ATLAS-2M at Week 48 Stratified by TDF Use at Baseline

<table>
<thead>
<tr>
<th>Outcome, n (%)</th>
<th>Switched to CAB+RPV LA</th>
<th>No Switch to CAB+RPV LA</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF at baseline</td>
<td>1694 (89.6)</td>
<td>1413 (85.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OCT at baseline</td>
<td>1291 (71.7)</td>
<td>2096 (131.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OCT at Week 48</td>
<td>1275 (70.8)</td>
<td>2085 (131.4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

and and treatment of treatment

541 RENAL/BONE OUTCOMES AFTER LONG-ACTING CABOTEGRAVIR + RILPIVIRINE IN ATLAS + ATLAS-2M

Paul Benn1, Piotr Budnik2, Sterling Wu3, Krischan J. Hudson1, Yuanquan Wang1, Ronald D’Amico1, Conn M. Harrington1, Susan L. Ford4, Rodica Van Solingen-Ricete1, Veerle Van Eygen5, Joseph W. Puli1, Kimberly Smith1, William R. Spreen1, ViiV Healthcare, Brentford, UK, 2GlaxoSmithKline, Collegeville, PA, USA, 3ViiV Healthcare, Research Triangle Park, NC, USA, 4GlaxoSmithKline, Research Triangle Park, NC, USA, 5Janssen, Beerse, Belgium

Background: Intramuscular cabotegravir (CAB) and rilpivirine (RPV) long-acting (LA) has been evaluated in two Phase 3 studies, ATLAS (NCT02951052) and FLAIR (NCT02938520), which demonstrated noninferiority of CAB+RPV LA dosed every 4 weeks (Q4W) to daily oral standard of care (SoC), and the Phase 3b study ATLAS-2M (NCT03299049), demonstrating noninferiority of CAB+RPV LA dosed every 8 weeks (Q8W) to Q4W. Tenofovir disoproxil fumarate (TDF) use is associated with renal/bone toxicities and improvements in renal/bone markers have been reported after cessation of TDF regimens. We present data from Week (W) 48 of ATLAS and ATLAS-2M examining changes in renal markers and bone turnover markers.

Methods: Data from ATLAS and ATLAS-2M were stratified by baseline (BL) TDF use. Efficacy and safety outcomes including changes in renal markers (urine protein-to-creatinine ratio [UPCR] and urine albumin-to-creatinine ratio [UACR]), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, procollagen 1 N-terminal propeptide [PINP], type 1 collagen C telopeptides [CTX]) were assessed. For ATLAS-2M, participants who transitioned from LA therapy in ATLAS, only data from ATLAS were included. W48 efficacy endpoints were proportion with plasma HIV-1 RNA ≥50 c/mL and <50 c/mL. Changes in bone markers were only available for ATLAS participants.

Results: In total, 1270 participants were included in the analysis; 665 were receiving TDF at BL and 605 were receiving non-TDF regimens. BL characteristics and outcomes at W48 are presented in Table 1. Within strata, the proportions of patients with HIV-1 RNA ≥50 c/mL and <50 c/mL were comparable across arms. Participants switching from TDF regimens to CAB+RPV LA experienced reductions in UPCR, compared with small increases observed among those switching from or continuing non-TDF regimens. Participants continuing TDF regimens had greater increases in UPCR (mean % UPCR change from BL: TDF: Q4W: +8.9; Q8W: +8.7; SoC: +8.0). In ATLAS, there were greater reductions in bone turnover markers in participants switching from TDF to CAB+RPV LA compared with other groups (mean change from BL in PINP and CTx [μg/L]: TDF: Q4W: –23.7; Q8W: –14.0; SoC: –12.4, 0.01; non-TDF: Q4W: –8.1, 0.06; SoC: –6.4, 0.07).

Conclusion: Participants switching from TDF to CAB+RPV LA experienced improvements in renal markers and bone turnover markers. These results support the therapeutic potential of CAB+RPV LA.

542 HIV IS ASSOCIATED WITH A HIGHER RATE OF COVID-19 DIAGNOSIS BUT NO ADVERSE OUTCOMES

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Background: Most available data on COVID-19 among persons with HIV (PWH) focuses on hospitalized patients, while COVID-19 risk among PWH relative to the general population remains inconclusive.

Methods: Using a retrospective comparative cohort analysis, we included all adults with established primary care service at UC San Diego who underwent testing for COVID-19 from March to July 2020. We dichotomized the cohort into two groups, PWH and non-PWH. We used bivariate analyses to compare group differences using a clinical hierarchical order, including COVID-19 diagnosis, hospitalization, intensive care unit (ICU) admission, intubation, and death. Logistic regression models for each hierarchical clinical outcome included pre-specified covariates (demographics, diabetes mellitus, and obesity) and additional covariates at a 0.20 threshold via backward model selection. Interactions between HIV status and other covariates were included at the 0.05 threshold. Additional covariates included insurance type, hypertension, cardiovascular disease, chronic kidney, lung, liver disease, rheumatologic disease, cancer history, ACE inhibitor or angiotensin II receptor blocker use, and active tobacco, alcohol or illicit drug use, and history of mental health disorder.

Results: Of 235609 participants, 3609 were PWH and 232000 non-PWH. Of them, 22% of PWH and 6% of non-PWH were tested positive for COVID-19. The PWH group had a higher proportion of younger individuals (76% vs 57%), males (85% vs 39%), non-whites (42 vs 35%), and a history of mental illness (58 vs 29%) than the non-PWH group. Of those tested, 7% of PWH and 2% of non-PWH tested positive for COVID-19. The adjusted odds ratio of COVID-19 diagnosis for HIV vs non-HIV was 4.32 (95% CI 3.09-6.04). No significant differences were observed for PWH compared to non-PWH in the proportion of patients hospitalized (15% vs 15%), admitted to ICU (7% vs 7%), requiring ventilation more frequently than those non-PWH (8% vs 3%), and mortality in any
models (see table); however, the limited number of events decreased the statistical power.

**Conclusion**: In our cohort, PWH were tested and diagnosed more frequently than those without HIV for COVID-19. PWH had an increased risk of becoming infected with COVID-19, even when adjusted for demographics and comorbidities. HIV status did not affect hospitalization, ICU admission, or mortality.

### Table 1: Adjusted Odds Ratios (95%CI) for outcome by predictor

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Death</th>
<th>Intubation</th>
<th>ICU</th>
<th>Hospitalization</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>4.32</td>
<td>0.44</td>
<td>2.89</td>
<td>1.51</td>
<td>7.64</td>
</tr>
<tr>
<td>Age per 10 years</td>
<td>0.73</td>
<td>0.71</td>
<td>1.61</td>
<td>0.44</td>
<td>0.30</td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>1.59</td>
<td>0.39</td>
<td>NS</td>
<td>0.36</td>
<td>3.75</td>
</tr>
<tr>
<td>Race (non-white vs. White)</td>
<td>0.96</td>
<td>2.56</td>
<td>1.02</td>
<td>0.84</td>
<td>4.80</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.43</td>
<td>2.97</td>
<td>3.20</td>
<td>4.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.60</td>
<td>1.50</td>
<td>3.20</td>
<td>4.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.51</td>
<td>0.51</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.42</td>
<td>0.43</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Drug use</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.26</td>
<td>0.32</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>0.34</td>
<td>0.35</td>
<td>0.24</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0.44</td>
<td>0.43</td>
<td>2.93</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Rheumatic disease</td>
<td>0.33</td>
<td>0.34</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>0.52</td>
<td>0.54</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic Kidney Disease</td>
<td>0.50</td>
<td>0.50</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.20</td>
<td>0.20</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mental health</td>
<td>0.73</td>
<td>0.73</td>
<td>0.50</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Conclusion**: The population frequency of COVID-19 detected in PWH was 1.4%, likely an underestimate of the true frequency of SARS-CoV-2 infection and COVID-19 disease due to evolving testing availability and access over time. A higher proportion of PWH with COVID-19 were Black or Hispanic, in excess of the overrepresentation of people of color with HIV compared to the general population. PWH with decreased eGFR, low CD4+ count, and obesity had greater risk of more severe COVID-19 disease. Our results highlight disparities in risk of COVID-19 acquisition among PWH in the US and indicate additional vigilance in screening and monitoring of COVID-19 among PWH with these characteristics. The expected accrual of additional COVID-19 cases will allow more precise evaluation of the impact of comorbidities.

### Table 2: Characteristic and clinical outcomes of hospitalized PWH and non-PWH in Spain during the first wave of the pandemic

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Not hospitalized</th>
<th>Hospitalized</th>
<th>RR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>300</td>
<td>246</td>
<td>54</td>
<td>1.02</td>
<td>0.55, 1.88</td>
<td>0.962</td>
</tr>
<tr>
<td>≥60</td>
<td>46</td>
<td>40</td>
<td>6</td>
<td>1.02</td>
<td>0.55, 1.88</td>
<td>0.962</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>490</td>
<td>444</td>
<td>46</td>
<td>1.00</td>
<td>0.52, 1.94</td>
<td>0.998</td>
</tr>
<tr>
<td>Black</td>
<td>50</td>
<td>43</td>
<td>7</td>
<td>1.00</td>
<td>0.52, 1.94</td>
<td>0.998</td>
</tr>
<tr>
<td>Other</td>
<td>47</td>
<td>48</td>
<td>0</td>
<td>0.21</td>
<td>0.01, 0.82</td>
<td>0.029</td>
</tr>
<tr>
<td>HIV transmission risk factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>397</td>
<td>359</td>
<td>38</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
<tr>
<td>MSM</td>
<td>100</td>
<td>90</td>
<td>10</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
<tr>
<td>COD</td>
<td>223</td>
<td>199</td>
<td>24</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
<tr>
<td>Lower CD4 count (cells/mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200</td>
<td>204</td>
<td>179</td>
<td>25</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
<tr>
<td>50-200</td>
<td>49</td>
<td>45</td>
<td>4</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
<tr>
<td>&lt;50</td>
<td>62</td>
<td>56</td>
<td>6</td>
<td>1.00</td>
<td>0.53, 1.96</td>
<td>0.999</td>
</tr>
</tbody>
</table>

**Conclusion**: COVID-19 cases & hospitalizations in a US multisite cohort of people with HIV

**Methods**: We examined all PWH with SARS-CoV-2 infection and COVID-19 disease identified from laboratory testing data (RT-PCR, antigen test results) and ICD-10 codes March-July 2020 from seven sites in the CFAR Network of Integrated Clinical Systems (CNICS) cohort. Cases were verified by medical record review. We evaluated predictors of increased disease severity, indicated by hospitalization. Relative risks were estimated using Poisson regression, adjusted for clinical and demographic characteristics using disease risk scores. The expected accrual of additional COVID-19 cases will allow more precise evaluation of the impact of comorbidities.

**Background**: COVID-19 outcomes among people with HIV (PWH) remain inconclusive. We characterized all cases of COVID-19 identified in a long-term multi-site cohort of PWH, as well as factors associated with increasing severity of COVID-19 during the early months of the COVID-19 pandemic.

**Methods**: We examined all PWH with SARS-CoV-2 infection and COVID-19 disease identified from laboratory testing data (RT-PCR, antigen test results) and ICD-10 codes March-July 2020 from seven sites in the CFAR Network of Integrated Clinical Systems (CNICS) cohort. Cases were verified by medical record review. We evaluated predictors of increased disease severity, indicated by hospitalization. Relative risks were estimated using Poisson regression, adjusted for clinical and demographic characteristics using disease risk scores.

**Results**: Among 13,862 PWH in care (20% female, median age 52 (IQR 40-59), 58% Black or Hispanic race/ethnicity), 198 COVID-19 cases were detected during the study period. A higher proportion of PWH with COVID-19 were female (27%), Black or Hispanic (76%), and had BMI ≥30 (45%). No significant differences in CD4+ count (current or lowest) were seen between PWH with and without COVID-19. We found evidence suggesting more unstable housing among COVID-19 cases compared to non-cases (14% vs. 9%). Among PWH with COVID-19, 38% (95% CI 0.29, 0.46) were hospitalized, 10% (5%) required intensive care, 8% (4%) received invasive mechanical ventilation, and 2% (4%) died. Hospitalization among PWH with COVID-19 was associated with: CD4+ count ≤350 (aRR 1.77; 95% CI 1.05, 2.98); age ≥60 (aRR 2.0; 95% CI 1.13, 3.54); pre-existing kidney disease with eGFR <60 (aRR 1.76; 95% CI 0.99, 3.13); and BMI ≥30 (aRR 1.96; 95% CI 1.02, 3.78) (Table).
Methods: HI admin were identified by reviewing clinical records and laboratory registries of 10,922 patients in active follow-up within the Spanish HIV Research Network (CoRIS) up to June 30, 2020. Each HI admin was matched with 5 HI neg of the same age and sex randomly selected from COVID-19 Spain, a multicenter cohort of 4,035 patients hospitalized with PCR confirmed COVID-19 in Spain (Clin Microbiol Infect 2020;26:1525-36). Data were collected with the ISARIC-WHO Core case report form (https://isaric.org/document/covid-19-crfr/). The COVID-19 SEIMC score (predictive of 30-day mortality), based on age, sex, dyspnea, O2 saturation, neutrophil-to-lymphocyte ratio, and estimated glomerular filtration rate, was calculated at admission in all patients (ESCMID Conference on Coronavirus Disease, 2020, Abstract#00513). Outcomes included the need for mechanical ventilation and all-cause in-hospital mortality.

Results: Forty-five patients with PCR confirmed COVID-19 were identified in CoRIS, 21 of which were hospitalized. A total of 105 age/sex-matched controls were selected from COVID-19 Spain. The median age in both groups was 53 (Q1-Q3, 46-56) years, and 90.5% were men. In HI-pos, 19.1% were IDUs, 95.2% were on ART, 94.9% had HIV RNA < 50 copies/mL, and the median (Q1-Q3) CD4+ count was 595 (349-798) cells/mm3. No statistically significant differences were found between groups in number and type of comorbidities, presenting signs and symptoms, laboratory parameters, and radiology findings. The median (Q1-Q3) COVID-19 SEIMC score on admission was 4 (2-7) and 5 (3-7) in HI-pos and HI neg, respectively; P=.89. Corticosteroids were administered to 33.3% and 27.4% HI-pos and HI neg, respectively; P=.58. Remdesivir was administered to 0 and 2.9% HI-pos and HI neg, respectively; P=.426. During admission, 9.5% HI-pos and 23.3% HI neg underwent mechanical ventilation; P=.158. In-hospital mortality was 9.5% in HI-pos and 11.4% in HI neg; P=0.800.

Conclusion: Our findings suggest that well-controlled HIV infection does not modify the clinical presentation or worsen clinical outcomes in patients hospitalized with COVID-19.

Table. Characteristics and outcomes of HI admin and HI neg hospitalized with COVID-19

<table>
<thead>
<tr>
<th>Variable</th>
<th>HI-pos N=21</th>
<th>HI neg N=105</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest X-ray on admission – No./with data (%)</td>
<td>20(95.2)</td>
<td>100(99.2)</td>
<td>&gt;.999</td>
</tr>
<tr>
<td>Presence of lung infiltrates</td>
<td>18 (90.0)</td>
<td>86 (80.0)</td>
<td>.631</td>
</tr>
<tr>
<td>Comorbidities – No./with data (%)</td>
<td>21/21 (100)</td>
<td>91/105 (86.7)</td>
<td>.124</td>
</tr>
<tr>
<td>None</td>
<td>8 (38.1)</td>
<td>36 (34.6)</td>
<td>.782</td>
</tr>
<tr>
<td>1-2</td>
<td>9 (42.9)</td>
<td>43 (40.5)</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>4 (18.6)</td>
<td>12 (11.3)</td>
<td></td>
</tr>
<tr>
<td>COVID-19 SEIMC score % – No./with data (%)</td>
<td>15/21 (71.4)</td>
<td>85/105 (80.5)</td>
<td>.377</td>
</tr>
<tr>
<td>Median (Q1-Q3)</td>
<td>4 (2-7)</td>
<td>5 (3-7)</td>
<td>.966</td>
</tr>
<tr>
<td>Corticosteroids – No./with data (%)</td>
<td>7/21 (33.3)</td>
<td>28/102 (27.4)</td>
<td>.586</td>
</tr>
<tr>
<td>Remdesivir – No./with data (%)</td>
<td>2/21</td>
<td>3/102 (2.9)</td>
<td>.426</td>
</tr>
<tr>
<td>Mechanical ventilation – No./with data (%)</td>
<td>2/21 (9.5)</td>
<td>24/103 (23.3)</td>
<td>.158</td>
</tr>
<tr>
<td>In-hospital mortality – No./with data (%)</td>
<td>2/21 (9.5)</td>
<td>12/105 (11.4)</td>
<td>.800</td>
</tr>
</tbody>
</table>

* Mortality risk: 0.2 low (0.1-3.7), 0.5 moderate (4.7-3.3), 4.9 high (16.4-18.6), 5.0 very high (27.7-100).

546 RISK FACTORS FOR HOSPITALIZATION IN PEOPLE WITH HIV AND COVID-19


University of Colorado Anschutz Medical Campus, Aurora, CO, USA; Denver Public Health, Denver, CO, USA; Denver Health Medical Center, Denver, CO, USA

Background: People living with HIV are thought to be at higher risk for poor outcomes (including higher hospitalization and mortality rates) from SARS-CoV-2 (COVID-19) infection. Whether risk is linked to HIV-related factors, demographics or comorbid burden is unclear. We examine risk factors and outcomes of those living with HIV who acquired COVID-19 and received care within two large healthcare systems in Denver, CO.

Methods: A retrospective analysis was conducted for all individuals with HIV diagnosed with COVID-19 at the two largest Colorado HIV care centers between 1 March and 31 October 2020. COVID-19 diagnosis required a positive PCR result; HIV diagnosis was extracted from the medical record. Risk factors for hospitalization included demographics and comorbidities.
hospitalization and longer hospital length of stay (LOS) were examined and compared via univariate and multivariable analysis.

**Results:** Among 94 patients, 81% were male, with a mean age of 46 (SD 13.5) years. The majority had HIV-1 RNA levels <50 copies/mL (87%) and CD4 count >500 cells/mm³ (55%). Most (75%) had ≥1 comorbidity; 64% were overweight or obese. 39% of patients were admitted to the hospital (71% to intensive care). Increased odds of hospitalization were associated with increased age, lower CD4 count, and increased number of comorbidities (including diabetes, hypertension, chronic kidney disease, chronic pulmonary disease, cardiac disease, mental health concerns, and obesity) (Table). In multivariable analyses, only lower CD4 count (OR 1.28) and comorbidity count (OR 1.62) remained significant. Among hospitalized patients, longer LOS was univariately associated with age (52% longer LOS per 10 year age increase [95% CI 16,101%], p = 0.004) but not CD4 count (-8% [95% CI -18, 5%]) change in LOS per 100 cell/mm³ increase, p = 0.21). HIV-1 viral load ≥200 copies/mL (-33% [95% CI -77, 90%], p = 0.44), or comorbidity count (10% [95% CI -17, 43%) change in LOS per additional comorbidity, p = 0.49). Sensitivity analyses excluding 9 patients hospitalized for non-COVID reasons provided similar findings.

**Conclusion:** Lower CD4 count was associated with an increased risk of hospitalization among patients with concurrent HIV and COVID-19, suggesting that successful HIV treatment remains a key component to decreasing HIV-related morbidity.

### Table: Odds of Hospitalization among 94 Adults with HIV and COVID-19

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Multivariable*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10-year increase)</td>
<td>1.04 (1.00, 2.17)</td>
<td>1.32 (0.90, 1.95)</td>
</tr>
<tr>
<td>Sex (Female vs Male)</td>
<td>1.03 (0.36, 2.96)</td>
<td>0.95</td>
</tr>
<tr>
<td>Race/ethnicity (refWhite)</td>
<td>2.30 (0.97, 5.45)</td>
<td>0.06</td>
</tr>
<tr>
<td>Primary Language (refEnglish)</td>
<td>0.23 (0.10, 1.25)</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (refUnderweight or normal)</td>
<td>2.84 (2.05, 3.70)</td>
<td>0.21</td>
</tr>
<tr>
<td>Overweight or obese</td>
<td>0.86 (0.28, 2.88)</td>
<td>0.36</td>
</tr>
<tr>
<td>CD4 count (per 100 cells/mm³ decrease)</td>
<td>1.32 (1.11, 1.56)</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV-1 RNA (ref ≤200 copies/mL) &gt;200 copies/mL</td>
<td>2.50 (0.74, 8.75)</td>
<td>0.14</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker (vs non-smoker)</td>
<td>0.07 (0.29,3.28)</td>
<td>0.46</td>
</tr>
<tr>
<td>Former smoker (vs non-smoker)</td>
<td>2.30 (0.86, 6.15)</td>
<td>0.12</td>
</tr>
<tr>
<td>Comorbidity Count</td>
<td>1.77 (1.23, 2.56)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Multivariable model included age, CD4 count, and comorbidity count

**547 COMORBIDITY BURDEN IS ASSOCIATED WITH HOSPITALIZATION FOR COVID-19 AMONG PWH**


1 Emory University, Atlanta, GA, USA, 2 Atlanta VA Medical Center, Decatur, GA, USA, 3 Grady Health System, Atlanta, GA, USA

**Background:** The contributions of non-AIDS comorbidities and HIV-related factors to coronavirus disease 2019 (COVID-19) outcomes among persons with HIV (PWH) remain unclear. We aimed to identify risk factors for COVID-19 hospitalization among PWH.

**Methods:** We evaluated all adult (≥18 years) PWH with a positive SARS-CoV-2 PCR evaluated in a public safety-net hospital system, a Ryan White-funded HIV clinic and a Veterans Affairs medical center in Atlanta, GA between March 1, 2020 and November 15, 2020. Demographic and clinical characteristics and COVID-19 disease outcomes were ascertained by medical record abstraction. We performed multivariable logistic regression to determine associations with COVID-19 hospitalization.

**Results:** 180 patients (mean age 49 years, 78% male, 78% Black, 14% Latina) were included. 97% were on antiretroviral therapy (ART), 91% had HIV-1 RNA <200 copies/mL, and mean CD4 count was 527 cells/mm³, 60 patients (33%) were hospitalized, 28 (47%) required supplemental oxygen. Overall mortality rate among PWH was 1.63%; mortality among hospitalized PWH was 5%. 130 patients (72%) had at least 1 non-AIDS comorbidity; 22% had >4 comorbidities (hypertension, dyslipidemia, obesity and diabetes were most prevalent). In univariable models, age, hypertension, dyslipidemia, diabetes, heart disease, and chronic kidney disease were associated with hospitalization. HIV characteristics including CD4 count, viral load, and ART use were not associated with hospitalization. After adjusting for these baseline characteristics associated with hospitalization, only age [aOR(95%CI) 1.073 (1.036-1.110), p<0.0001] and diabetes mellitus [aOR(95%CI) 2.653 (1.027-6.833), p=0.0439] were associated with hospitalization. In a multivariable model adjusting only for age, comorbidity count was associated with a 25% increased risk for hospitalization [aOR(95% CI) 1.245 (1.013-1.531), p=0.0375], and having >4 comorbidities was associated with a 2.8-fold increased risk of hospitalization compared with 0-1 comorbidities [aOR(95% CI) 2.848 (1.174-6.910), p=0.0240] (Figure). In age-adjusted analyses restricted to CD4 <200 cells/mm³ or HIV-1 RNA >200 copies/mL, HIV-related factors were not associated with hospitalization.

**Conclusion:** In a cohort of PWH with well-controlled HIV and COVID-19, age and non-AIDS comorbidities, but not HIV-related factors, were associated with hospitalization for COVID-19. Further research into causes of severe COVID-19 among PWH is warranted.
and mechanical ventilation but were not significantly more at risk of death at 30 days after COVID-19 diagnosis compared to non-HIV controls.

Table 1. Comparison of demographic characteristics, comorbidities, presenting symptoms, laboratory parameters and clinical outcomes among HIV and non-HIV COVID-19 patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV (N=1036)</th>
<th>Non-HIV (N=29556)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>43.34 ± 13.59</td>
<td>46.4 ± 18.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1137 (69.4%)</td>
<td>13089 (44.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>African American or Hispanic</td>
<td>1102 (67.3%)</td>
<td>10013 (33.97%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>767 (46.6%)</td>
<td>7717 (25.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td>404 (24.7%)</td>
<td>4368 (14.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cough</td>
<td>297 (18.2%)</td>
<td>4547 (16.9%)</td>
<td>0.1519</td>
</tr>
<tr>
<td>Fever</td>
<td>184 (11.9%)</td>
<td>2480 (9.3%)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Hemoglobin, g/dl (mean ± SD)</td>
<td>12.8 ± 2.5</td>
<td>12.0 ± 2.2</td>
<td>0.0006</td>
</tr>
<tr>
<td>Procalcitonin, ng/ml (mean ± SD)</td>
<td>2.5 ± 10.1</td>
<td>13.5 ± 9.9</td>
<td>0.042</td>
</tr>
<tr>
<td>Interleukin-6, pg/ml (mean ± SD)</td>
<td>258 ± 642</td>
<td>98 ± 249</td>
<td>0.510</td>
</tr>
<tr>
<td>Intensive care and mechanical ventilation</td>
<td>49 (2.4%)</td>
<td>4604 (1.6%)</td>
<td>0.095</td>
</tr>
<tr>
<td>Mortality at 30 days</td>
<td>47 (2.5%)</td>
<td>6701 (2.3%)</td>
<td>0.142</td>
</tr>
</tbody>
</table>

549 PREVALENT FACORS ASSOCIATED WITH SARS-COV-2 INFECTION IN A SPANISH HIV COHORT
Juan Berenguer, Cristina Díez, María Martín-Vicente, Rafael Micani, María Jesús Pérez-Elias, Lucio Jesús F. García-Fraile, Francesc Vidal, Inés Suárez-García, Daniel Podzamczer, Juan C. López, José R. Arribas, Santiago Moreno, Juan González-García, Salvador Resino, Immaculada Jarrín, Hospital General Universitario Gregorio Marañón, Madrid, Spain, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain, La Paz University Hospital, Madrid, Spain, Hospital Ramón y Cajal, Madrid, Spain, Hospital Universitario La Princesa, Madrid, Spain, Hospital Universitario de Tarragona Joan XXIII, Tarragona, Spain, Hospital Universitario Infanta Sofia, San Sebastián de los Reyes, Spain, Hospital Universitario de Bellvitge, Barcelona, Spain, Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid, Spain

Background: Within a prospective cohort of people with HIV (PWH) in Spain, we assessed the prevalence of SARS-COV-2 antibodies (Ab), the proportion of asymptomatic COVID-19, and identified predictors of infection.

Methods: We determined SARS-COV-2 Ab in plasma samples collected from April 1st to September 30th, 2020, from enrollees in the Spanish HIV Research Network Cohort (CoRIS), a prospective national cohort of PWH, naïve to ART at study entry, seen for the first time from January 1st, 2004. Samples were stored at -80°C in the Spanish HIV Biobank, and serology was performed using the Platelia SARS-CoV-2 Total Ab assays (BioRad, Hercules, CA, USA). Illness severity (NIH criteria) was assessed by medical records review and, if needed, participant interviews. Multivariable logistic regression analysis was used to identify predictors of seropositivity among the following variables: sex, age, country of birth, education level, comorbidities (hypertension, chronic heart disease, diabetes, non-AIDS related cancer, chronic kidney disease, cirrhosis), route of HIV acquisition, ART, CD4+ cell count, HIV viral load, and CD4+ cell count loss. The seroconversion rate was calculated using the Kaplan-Meier method.

Results: During the study period, blood samples were collected and stored in the HIV Biobank from 1,076 consecutive PWH in CoRIS: 88.0% male at birth, 91.4% undetectable HIV viral load. SARS-COV-2 Ab were detected in 91 PWH, 91.4% undetectable HIV viral load. SARS-CoV-2 Ab were detected in 91 PWH, 4.4%, severe in 3 (3.3%), and 0 critical. Seven PWH (7.7%) were hospitalized.

Conclusion: A large proportion of SARS-COV-2 infections among PWH were asymptomatic. Birth in LA countries and arterial hypertension were associated with increased risk of SARS-COV-2 seropositivity. Our analysis, adjusted by comorbidities and other variables, suggest that TDF/FTC may prevent SARS-COV-2 infection among PWH.

Table. Variables independently associated with COVID-19 seropositivity (Ab+) among 1,076 PWH

<table>
<thead>
<tr>
<th>Country of birth</th>
<th>Ab+ (% of total)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>45/764 (7.5%)</td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Latin American Countries</td>
<td>23/231 (14.4%)</td>
<td>2.16 (1.36 - 3.4)</td>
<td>&lt;0.001</td>
<td>2.3 (1.42 - 3.85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>4/95 (4.2%)</td>
<td>0.60 (0.21 - 1.68)</td>
<td>.328</td>
<td>0.64 (0.22 - 1.88)</td>
<td>.419</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>56/740 (7.5%)</td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35/328 (10.7%)</td>
<td>1.45 (0.92 - 3.31)</td>
<td>.086</td>
<td>1.63 (1.03 - 2.67)</td>
<td>.290</td>
</tr>
</tbody>
</table>

550 MYOCARDIAL INJURY AS A PREDICTOR OF MORTALITY AND ADVERSE OUTCOMES IN COVID-19
Gavin Mannmahan, Debbie Falconer, Zakeen Abdi, Samuel Conway, Athanasios Kosovitsas, Alan Hunter, Callum Little, Sabine Kinloch-de Loes, Colette Smith, Margarita A. Johnson, Roby D. Rakshit
Royal Free Hospital, London, UK, University College London, London, UK

Background: The American College of Cardiology suggested physicians should only measure troponin and brain natriuretic peptide (BNP) if myocardial infarction or heart failure were suspected in people with COVID-19. We aimed to evaluate the use of biomarkers on admission to hospital and the impact on mortality and morbidity.

Methods: Consecutive patients presenting with COVID-19 (reverse transcription PCR positive) between Feb 27-May 2020 were included in this retrospective, observational, single-center study. Clinical information was collected on admission and during hospitalization by physicians and later analysed by specialist cardiology registrars. 1675 patients were PCR+ve with 1036 having a high sensitivity troponin (hsTroponin T) on admission. 3735 (35.8%) patients were hsTroponin T negative (<15ng/L) and 664 (46.1%) had evidence of myocardial injury on admission (hsTroponin T ≥15ng/L). Subsequently demographic details were compared, as well as primary outcomes of death, ICU admission and COVID severity. Secondary outcomes were ARDS, myocardial infarction (MI); comparison with other biomarkers: NT-proBNP, d-dimer, CRP, LDH and fibrin.

Results: Demographic data revealed no significant increase in proportions of Black, Asian or ethnic minorities in the myocardial injury group, however, patients were older (74.9±13.5 vs 54.7±13.7yrs; p<0.001) and had significantly more co-morbidities such as diabetes (37 vs 13%), hypertension (34 vs 29%), ischemic heart disease (16 vs 2%), other cardiac conditions (59 vs 5%), malignancy (11 vs 1%), COPD (9 vs 4%), CKD stage ≥3 (40 vs 3%)(p<0.001). Mortality was significantly higher in the myocardial injury group, 3024 (45.5%) vs 297 (7.8%)(p<0.001), as were secondary outcomes of critical COVID (47 vs 19%)(p<0.001), ARDS (20 vs 4%; p<0.001), Type 1 MI (16.6 vs 0.3%; p<0.01) and Type 2 MI (44 vs 26%; p<0.001). Interestingly, ICU admission (19 ± 23%)(p<0.09), pulmonary embolism (11 ± 6%)(p=0.22), stroke (1 ± 0.5%; p=0.05) did not reach significance. Analysis of bio-markers on admission (Fig 1) demonstrated hs Troponin T (AUC 0.75 CI 0.69-0.81) and NT-proBNP (AUC 0.75 CI 0.69-0.81) had more sensitivity 83%;85% and specificity 52%;58%, respectively at predicting death than d-dimer, CRP, LDH and fibrin.

Conclusion: Early detection of elevated hs Troponin T and NT-proBNP predicts mortality and morbidity in patient with COVID-19. Routine measurement of cardiac biomarkers should be considered in patients with COVID-19 at the time of hospital admission in order to optimise risk stratification and guide monitoring.
551 HIGH RATE OF PERSISTENT SYMPTOM 4 MONTHS AFTER COMMUNITY-MANAGED COVID-19 INFECTION

David Darley, Greg Dore, Lucette A. Cysique, Jeff Masters, Anthony Byrne, Bruce Brew, Philip Cunningham, Anthony Kelleher, Gail Matthews, for the ADAPT Study Group
St Vincent’s Hospital, Sydney, Australia

Background: The spectrum of recovery following community-managed and hospitalized SARS-CoV-2 infection remains uncertain. The aim of the ADAPT study was to determine prevalence and nature of persistent symptoms after community and hospitalised SARS-CoV-2 infection; and to evaluate lung function; health-related quality of life (HRQOL); and neurocognitive abnormalities.

Methods: A prospective observational cohort study was performed at St Vincent’s Hospital Sydney Australia. Adult patients with a positive SARS-CoV-2 RNA PCR test between Mar-2020 and Apr-2020 including mild, moderate, and severe acute infection were offered enrollment. The clinical outcomes included symptom prevalence at initial infection and follow-up, HRQOL measures, pulmonary function, neurocognition and COVID-19 antibody responses. Initial study assessments were performed up to 4 months after first detection of SARS-CoV-2.

Results: Ninety-six patients were recruited following community-managed mild (39%) and moderate (50%), and hospitalized severe (11%) COVID-19 infection. 39.7% patients had persistent symptoms at median 72 days after diagnosis (IQR 65-87), including those in severe (77.8%), moderate (33.3%), and mild (36.7%) sub-populations. The most common persistent symptoms were fatigue (28%), shortness of breath (25%) and cough (21%). Total lung capacity (TLC) was significantly lower after severe, compared with community-managed, COVID-19, p=0.05. Abnormal diffusion capacity for carbon monoxide values were observed in 16% patients unrelated to acute illness severity. Twenty-four percent patients demonstrated anxiety/depression, as measured by SPHERE-34, CRP at admission, Creatinine at admission, LDH at Admission, Femoral at Admission

Conclusion: A considerable proportion of patients experience persistent symptoms at 4 months after SARS-CoV-2 infection including one third of community managed patients. High rates of depression and anxiety were reported across the cohort. Outpatient follow-up to further assess those with persistent symptoms after COVID-19 is important to allow multi-disciplinary input, further investigation, and appropriate management. Data collection on the prevalence of persisting symptoms at 8 month follow-up of the ADAPT study is currently underway.

552 COGNITIVE DEFICITS ARE NOT A BYPRODUCT OF ANXIO-DEPRESSIVE SYMPTOMS IN COVID-19

Lucette A. Cysique, Yasmin Allen-Davidian, David Darley, Anthony Byrne, Kay Wilhelm, Greg Dore, Gail Matthews, Bruce Brew

1University of New South Wales, Sydney, Australia, 2Macquarie University, North Ryde, Australia, 3St. Vincent’s Hospital, Sydney, Australia, Kirby Institute, Sydney, Australia

Background: Cognitive deficits and anxio-depressive symptoms have been described in the recovery phase of COVID-19. Their association, or lack thereof, may assist in better understanding the long-term consequences of COVID-19.

Methods: Patients underwent neurocognitive and mental health assessment at 2 months after initial SARS-CoV-2 infection as part of the St Vincent’s Hospital ADAPT study, a prospective cohort study after COVID-19 disease. Cognition was assessed with the culture fair computerized Cogstate battery. A demographically-corrected composite z-score was created representing global cognitive performance, and then classified as impaired, borderline, and unimpaired. Anxiety-depressive symptoms were assessed with the Depression in the Medical III scale-10 (DMI-10), the Somatic and Psychological HEalht Report-34 (SHIPERE) Psych subscale, and the Impact of Events Scale-Revised (IESR). The scales scores were amenable to a single Principal Component explaining 80% of the variance. Female sex (p<.01) and Non-English-Speaking Background-NESB (p=.02) were associated with greater anxiety-depressive symptoms but not age, education. Regression analyses tested sex and NESB unadjusted and adjusted predictions of anxiety-depression to cognition.

Results: 132 patients (mean age=46±15; 40% women, median education=16 years, 10% NESB) were tested after predominantly community-managed COVID-19 (10% hospitalised). 17% had impaired global cognition, and 10% had borderline deficit. 25% had elevated symptoms on the DMI-10 (score>9), 13% on the IESR (score>24) and 35% on the SPHERE Psych scale (score≥2). Anxiety-depression was not predictive of cognitive performance (unadjusted p=.43; adjusted p=.98) and of impaired/unimpaired status (unadjusted p=.50; adjusted p=.78). Anxiety-depression tended to predict of borderline (vs. unimpaired) performance in unadjusted (p=.08) and adjusted (p=.09) analyses. This was explained by the fact that women who had borderline performance tended to report higher anxiety-depressive symptoms compared to their peers who were unimpaired (p=.06); further impaired women (vs. unimpaired) tended to report the least anxiety-depressive symptoms (p=.09).

Conclusion: Cognitive deficits are not a by-product of anxiety-depressive symptoms in recovering COVID-19 patients. Women appear to have a higher degree of introspection and reaction to very mild cognitive decline. Cognitive changes appear to be a direct consequence of COVID-19.

553 EVALUATING FACTORS MEDIATING THE RELATIONSHIP BETWEEN MALE SEX AND COVID-19 SEVERITY

Randy Stalter, Vidya Attur, Fan Xia, Katherine K. Thomas, Kristine Lan, Alex Greninger, Rena Patel

1University of Washington, Seattle, WA, USA, 2National Alzheimer’s Coordinating Center, Seattle, WA

Background: Males have experienced higher rates of severe COVID-19 outcomes compared to females but the underlying causal mechanisms of this relationship are not well understood. We leveraged existing electronic medical records (EMR) to evaluate associations between sex and COVID-19 test positivity, disease severity, viral burden, and death, and assess factors that mediate the relationship between male sex and severe COVID-19 disease.

Methods: We conducted a retrospective cohort study with data collected from University of Washington Medicine EMR from March 1 to September 29, 2020. All persons, regardless of age, were included if they had a conclusive diagnostic COVID-19 PCR test result. We defined severe COVID-19 disease as a score >5 on the WHO clinical progression scale. We used Poisson regression to assess sex differences in risk for COVID-19 test positivity, disease severity and COVID-19 related death, and linear regression to compare viral cycle threshold at the first positive test. We conducted mediation analyses to assess interventional indirect effects of male sex on severe COVID-19 risk through socioeconomic status (SES, based on area deprivation and insurance type), comorbidities, and inflammation status. Models controlled for age and race/ethnicity.

Results: Of individuals with SARS-CoV-2 testing records, 32,919 males and 34,733 females had a conclusive PCR test during our observation period. Males were 13% more likely to test positive than females in multivariable analysis (RR=1.13; 95% CI: 1.04-1.24; Table). Males had 85% higher risk for severe COVID-19 disease (RR=1.85; 95% CI: 1.33-2.62) and 66% higher risk for COVID-19
related death (RR=1.66; 95% CI: 0.95-2.98) than females following a positive test result. No difference was observed in cycle threshold at first positive test between males and females (p=0.69). Mediation analyses indicated a significant interventional indirect effect of male sex on severe COVID-19 disease through inflammation status (RR=1.07; 95% CI: 1.01-1.13), and less so through SES or comorbidities.

**Conclusion:** In our cohort, males had higher test positivity and greater risk of COVID-19 severity and death. This relationship between male sex and severe COVID-19 seems to act in part through inflammation status. Additional analyses in larger cohorts are needed to better understand the full range of socio-behavioral and biologic factors that mediate the relationship between sex and poor COVID-19 outcomes.

### Table 1: Persistent symptoms and overall severity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Initial Hospitalization</th>
<th>3 months</th>
<th>6 months</th>
<th>1 and 6 months</th>
</tr>
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<tbody>
<tr>
<td><strong>Cardiorespiratory Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>44 (77.4%)</td>
<td>42 (69.1%)</td>
<td>33 (69.6%)</td>
<td>31 (75.0%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>31 (54.5%)</td>
<td>44 (70.4%)</td>
<td>48 (89.8%)</td>
<td>73 (87.5%)</td>
</tr>
<tr>
<td>Myalgia or Arthralgia</td>
<td>144 (28.8%)</td>
<td>54 (11.1%)</td>
<td>64 (17.8%)</td>
<td>18 (4.8%)</td>
</tr>
<tr>
<td>Anosmia or Ageusia</td>
<td>21 (3.7%)</td>
<td>5 (1.0%)</td>
<td>3 (0.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Neuropsychiatric Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea or Vomiting</td>
<td>112 (19.8%)</td>
<td>98 (20.1%)</td>
<td>88 (24.2%)</td>
<td>21 (7.4%)</td>
</tr>
<tr>
<td>Headache</td>
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<td>24 (4.6%)</td>
<td>34 (9.9%)</td>
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</tr>
<tr>
<td>Insomnia</td>
<td>21 (1.3%)</td>
<td>29 (5.7%)</td>
<td>20 (5.9%)</td>
<td>4 (1.4%)</td>
</tr>
<tr>
<td>Sleep disturbances</td>
<td>32 (5.0%)</td>
<td>24 (5.3%)</td>
<td>21 (5.8%)</td>
<td>2 (0.7%)</td>
</tr>
<tr>
<td>Depression or Anxiety</td>
<td>2 (0.9%)</td>
<td>17 (3.5%)</td>
<td>18 (5.1%)</td>
<td>3 (1.1%)</td>
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<td>12 (4.9%)</td>
<td>3 (1.1%)</td>
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<tr>
<td><strong>Gastrointestinal Symptoms</strong></td>
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<td></td>
</tr>
<tr>
<td>Nausea or Vomiting</td>
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</tr>
<tr>
<td>Abdominal Pain</td>
<td>124 (21.9%)</td>
<td>20 (3.5%)</td>
<td>21 (5.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Diarrhea</td>
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**Long-Term Sequela of SARS-CoV-2 Infection in a Retrospective New York City Cohort**

Sherif Shoucri, Matthew A. Adani, Lawrance Purpura, Clare DeLaurentis, Deborah Theodore, Michael T. Yin, Magdalena Sobieszczyk, Delivette Castor, Jason Zucker, for the Columbia Longitudinal COVID Group

**Background:** The long-term sequelae of coronavirus disease 2019 (COVID-19) have been increasingly recognized. Cardiac, pulmonary, and neuropsychiatric symptoms have been reported to persist up to two months after hospitalization. However, much remains to be learned about the durable long-term effects of COVID-19 for patients and the health care system. Here, we describe the persistence of COVID-19 sequelae up to six months after presentation.

**Methods:** We examined the electronic medical records of the first 1190 patients diagnosed with SARS-CoV-2 infection by reverse transcription polymerase chain reaction assay and hospitalized at a quaternary-care center in New York City. All initial hospital presentations occurred between March 1 and April 8, 2020. We manually abstracted data for two follow-up periods representing three- and six-months post-hospitalization. Abstracted information included type and dates of encounters; use of tele-health; presence and persistence of COVID-19 sequelae up to six months after presentation.

**Results:** Patients had a median age of 60 and 61 years at three and six months, respectively. About 45% were female and 50% identified as Hispanic/Latinx. Of 1190 patients, 78% (N=928) survived their initial hospitalization. Among the 61% (n=570) of survivors who followed up encounters at three and six months, patients frequently reported cardiopulmonary symptoms (35.7% and 28%), dyspnea (22.1% and 15.9%), generalized symptoms (25.4% and 26.4%) and neuropsychiatric symptoms (20.1% and 24.2%). Tele-health encounters represented 59% and 28.2% of encounters at three and six-months, respectively. Twenty-percent of patients had reduced mobility or reduced independence in the six months after hospitalization. Of survivors, 17% were discharged to a nursing or rehabilitation facility and 10.3% remained there at three months post-hospitalization.

**Conclusion:** The prevalence was high of at least one COVID-associated symptom six months after hospitalization. Cardiopulmonary symptoms were most common and persisted longer than previously reported. Providers, patients, and their families must be sensitized to and anticipate these potential sequelae. Further follow-up and studies of COVID-19 survivors are necessary to confirm these findings and investigate outcomes beyond six months.
with symptom severity or risk for disease progression. The range of viral RNA shedding was remarkably similar across the range of symptom severity, suggesting symptom severity may not correlate with transmission risk or the potential to respond to antiviral therapy. Outpatient trials aimed at evaluating antiviral activity of new agents should focus enrollment on participants with recent onset of symptoms.

Figure. Scatterplots of nasopharyngeal SARS-CoV-2 RNA with A) days from symptom onset to study entry and B) total targeted symptom scores.

556 ROUTINIZATION OF TB INFECTION SCREENING WITH CD4 AND VIRAL LOAD MONITORING IN BRAZIL

Leila H. Chaisson1, Paula Travassos2, Silvia Cohn3, Solange Cavalcante4, Valeria Saraceni5, Renata Sperner-Gomes6, Leda F. Jamal6, Ana P. Loich1, Jose V. Madruga1, Marcelo Cordeiro-Santos7, Jonathan Golub8, Betina Duruom, for the PREVINE-TB Research Group1

1University of Illinois at Chicago, Chicago, IL, USA, 2Secretaria Municipal de Saúde do Rio de Janeiro, Rio de Janeiro, Brazil, 3The Johns Hopkins University, Baltimore, MD, USA, 4Instituto Nacional de Infectologia Enrondo Chagas, Rio de Janeiro, Brazil, 5Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil, 6Centro de Referência e Treinamento DST/AIDS-SE San Paulo, Brazil

Background: In Brazil, annual screening for latent tuberculosis infection (LTBI) via tuberculin skin testing (TST) is recommended for people with HIV (PWH) with CD4 > 350 cells/µl to guide tuberculosis preventive therapy (TPT). However, screening remains infrequent and TPT is markedly underutilized. We hypothesized that a strategy pairing screening via interferon-gamma release assay (IGRA) with routine CD4 and viral load (VL) monitoring could improve LTBI screening and TPT uptake for PWH.

Methods: PREVINE-TB is a multi-center study in four clinics in Rio de Janeiro, Manaus, and Sao Paulo, Brazil, with the overall objective of improving LTBI evaluation and TPT uptake for PWH. We implemented a strategy to integrate IGRA testing (via QuantiFERON-TB Gold Plus, QFT+) at routine CD4/VL blood draws. We enrolled PWH presenting for routine clinic or laboratory visits who were eligible for LTBI evaluation according to Brazilian national guidelines (CD4 > 350 or CD4 unknown; no negative TST within 12 months; no history of a positive TST, TPT, or TB treatment). Clinicians were trained to order IGRA, with blood draws scheduled for the patient’s next routine CD4 and/or VL test. To assess IGRA uptake during the 6-month PREVINE-TB pilot period, we determined the proportions of patients enrolled, referred for IGRA, and completing IGRA; and LTBI prevalence among those tested.

Results: From Jun-Nov 2020, we screened 1,504 PWH with CD4 > 350 or CD4 unknown and enrolled 665 (44%) eligible for IGRA, including 388 (58%) presenting to the laboratory and 277 (42%) presenting for clinic appointments. Of 839 not enrolled, 421 (50%) were ineligible for IGRA (including 124 who previously received TPT and 263 previously treated for active TB) and 314 (37%) were not referred by clinic physicians. Among 665 enrolled, we excluded 20 (3%) with incomplete data. Among 645 included, median age was 44 years (IQR 35-55) and 168 (26%) were female. IGRA was ordered for 642 (99.5%) and paired with a CD4 and/or VL order for 580/642 (90%). At the time of analysis, 491 (77%) patients had completed IGRA and 151 (23%) were scheduled for testing at a later date. 104/491 (21%) were IGRA-positive, including 59/185 (32%) in Rio de Janeiro, 24/133 (18%) in Manaus, and 20/173 (12%) in Sao Paulo.

Conclusion: Prevalence of LTBI among PWH receiving routine HIV care in three cities in Brazil was high, particularly in Rio de Janeiro. Routinely linking IGRA to CD4 and/or VL may increase known LTBI status, potentially leading to increased TPT.

557 ASSESSMENT OF TUBERCULOSIS DISEASE ACTIVITY IN MTB-INFECTED HIV PATIENTS OVER TIME

Inge Kroid1, Mohamed I. Ahmed2, Sacha Horn3, Christina Polya4, Allasha Eiber5, Ajay Parikh5, Leigh A. Eller6, Hannah Kibuuka1, Michael Hoelscher5, Anna S. Madhavan7, Jose Owothu8, Rebecca Loose9, Michael Hoelscher1, Julie Ake9, Christof Goldmacher9,4

1University Hospital LMU Munich, Munich, Germany, 2UIM Munich, Munich, Germany, 3US Military HIV Research Program, Silver Spring, MD, USA, 4U.S. Military HIV Research Program, Silver Spring, MD, USA, 5Makerere University Walter Reed Project, Kampala, Uganda, 6U.S. Military HIV Research Program, Munich, Germany

Background: HIV-infected patients in sub-Saharan Africa are at high risk of developing active tuberculosis (aTB). Studies assessing aTB disease activity and transmissibility over long periods in Mycobacterium tuberculosis (MTB) infected people living with HIV (PLWH) are lacking and difficult to conduct. Previous studies demonstrated that phenotypic characteristics of MTB-specific CD4 T cells – in particular expression of activation and maturation markers – detects aTB with high accuracy.

Methods: During the African Cohort Study (AFRICOS) Tuberculosis sub-study, 2024 East African PLWH from Kenya (Kisumu and South Rift Valley) and Uganda (Kayunga,) were subjected to molecular detection of MTB during yearly intervals from 2013 to 2017. Longitudinal PBMC sample sets from 46 patients were selected into three groups matched for age, sex and ART status; patients with clinically latent MTB infection was defined by continuous MTB sputum-negativity and absence of aTB symptoms; aTB patients were sputum MTB+ at baseline and initiated TB treatment. Incipient TB cases were unsuspicous for aTB at enrolment and developed sputum MTB+ aTB during the course of the AFRICOS study. In total 242 visits and 1556 person months were analyzed for MTB-specific T cell activation and maturation using intracellular cytokine staining as a surrogate marker of aTB disease activity.

Results: CD8 expression on MTB-specific T cells, but not bulk CD4 T cells, specifically differentiated active aTB from LTBI (p < 0.001) and was reduced upon TB treatment initiation (p < 0.001). 67% and 23% of incipient TB cases had activated MTB-specific T cells at 6 and 12 months before aTB diagnoses, respectively. Transient flair ups of MTB-specific T cell activation were also often observed in the majority of HIV patients with LTBI and after the end of TB treatment. These typically resolved without Isoniazid treatment. However, recurrent aTB was associated with persistent/recurrent MTB-specific T cell activation after the end of treatment.

Conclusion: The majority of HIV patients with clinically latent MTB infection experience periods of sub-clinical disease that either are spontaneously controlled or progress to aTB. Subclinical TB disease activation begins between 6 to 12 months prior to the sputum-based diagnoses of aTB in the majority of incipient TB cases. After the end of TB treatment, persistent or recurrent MTB-specific T cell activation is associated with recurrent aTB and/or treatment failure.
M. TUBERCULOSIS EXOSOME DETECTION FOR TB DIAGNOSIS IN CHILDREN LIVING WITH HIV

Sylvia LaCourse, Wenshu Zheng, Jaclyn Escudero, Lisa Cranmer, Irene Njuguna, Dalton Wamalwa, Elizabeth Maleche-Obimbo, Christopher Lyon, Grace John-Stewart, Tony Hu

1 University of Washington, Seattle, WA, USA, 2 Tulane University, New Orleans, LA, USA, 3 Emory University, Atlanta, GA, USA, 4 Kenyatta National Hospital, Nairobi, Kenya, 5 University of Nairobi, Nairobi, Kenya

Background: Non-sputum-based diagnostics for pediatric TB detection and treatment response are urgently needed for children living with HIV (CLHIV) who have high TB morbidity and mortality and are often missed by respiratory sampling. Exosomes are small extracellular vesicles (EVs) secreted by cells originating from endosomal cell compartments. We developed a nanoplasmon-enhanced scattering (nPES) assay which detects and quantifies M. tuberculosis-specific markers (LprG and lipoarabinomannan (LAM)) in EVs (Mtb-EVs) using as little as 1 μL of plasma.

Methods: Cryopreserved plasma from hospitalized children enrolled in a trial (NCT02063880) of urgent (<48 hrs) vs. post-stabilization (7-14 days) ART was evaluated by Mtb-EV nPES assay. Children underwent baseline TB investigations including sputum or gastric aspirates Xpert and culture, and stool Xpert. Plasma was collected at baseline, 2, 4, 12, and 24 weeks when possible. Children were classified per NIH pediatric TB diagnostic criteria as confirmed (Xpert and/or culture positive), unconfirmed (>2 of either signs/symptoms suggestive of TB, abnormal CKR, TB exposure or tuberculin skin test positive, or having positive TB treatment response), or unlikely TB.

Results: Among 72 children, 60% were male, median age was 1.4 years (IQR 0.6-3.7), 80% were severely immunosuppressed (WHO criteria), and 31% died. Ten percent (7/72) had confirmed, 50% (36/72) unconfirmed, and 40% (29/72) had unlikely TB. Twenty-four initiated TB treatment, with 14 (58%) considered to have treatment response. Mtb-EV nPES sensitivity was 86% (6/7) among confirmed and 72% (26/36) among unconfirmed TB cases. Specificity was 48% (14/29) among unlikely TB cases; but increased to 78% (7/9) among unlikely TB cases without symptoms. Mean Mtb-EV signals were higher among confirmed vs. unlikely TB overall and vs. unlikely TB without symptoms (p<0.05) and among unconfirmed TB vs. unlikely TB overall and vs. unlikely TB without symptoms (p<0.03) (Figure). Mtb-EV concentration decreased following TB treatment initiation in children with available longitudinal samples among 100% (4/4) CLHIV with confirmed TB and 67% (8/12) of unconfirmed TB cases with clinical improvement.

Conclusion: Mtb-EVs detected by nPES is a promising means of TB detection and monitoring of treatment response using non-respiratory sample which requires minimal blood volume in CLHIV. Detectable Mtb-EVs in symptomatic immunocompromised CLHIV without microbiologic confirmation may indicate TB missed by respiratory sampling.

SERUM MARKERS AND INTEGRATIVE MULTI-OMICS OF TB DIAGNOSIS IN ADVANCED HIV

Sonya Krishnan, Artur T. Queiroz, Amita Gupta, Nikhil Gupte, Gregory P. Bisson, Johnstone Kumwenda, Kogielem Naadoo, Lerato Mohapf, Vidy Mave, Rosie Mengibisa, Javier R. Lama, Mina C. Hosseinipour, Bruno B. Andrade, Petros Karakousis, for the ACTG A5274 REMEMBER and NWCS 414 Study Team

The Johns Hopkins University School of Medicine, Baltimore, MD, USA, Instituto Gonçalo Moniz, Salvador, Brazil, University of Pennsylvania, Philadelphia, PA, USA, University of Malawi, Blantyre, Malawi, University of Leeds-Zaka-Natal, Durban, South Africa, University of the Witwatersrand, Johannesburg, South Africa, D’Bryanne Jeejeebhoy Government Medical College, Pune, India, Durban International Clinical Research Site, Durban, South Africa, Associate Clinical Impacta Salud y Educacion, Barranquilla, Colombia, 9 University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Tuberculosis (TB) accounts for a large burden of morbidity and mortality among persons living with HIV (PLWH). Conventional methods of TB diagnosis, including smear microscopy and Xpert MTB/RIF have lower sensitivity in PLWH. Novel high-throughput approaches, such as miRNA and metabolomics, may advance our insights into subclinical and difficult to diagnose TB, especially in very advanced HIV.

Methods: We conducted a case-control study leveraging REMEMBER, a multi-country, open-label randomized controlled trial comparing 4-drug empiric TB treatment with isoniazid preventive therapy in PLWH initiating ART with CD4 cell counts <50 cells/µL. Active TB was ruled out at baseline. A total of 23 Cases (incident TB within 48 weeks post-ART initiation) were site-matched with up to 2 Controls. We performed miRNA next generation sequencing (qIAQEN), liquid chromatography-mass spectrometry quantitative metabolomic analysis (Metabolon, Inc.), and multiplex immunoassays (Luminex) on serum samples obtained at time of TB diagnosis. Multi-omics data were integrated, and the decision tree algorithm was used to identify the best model for TB diagnosis. The accuracy was measured by receiver operating characteristic (ROC) curve and area under the curve (AUC).

Results: The majority of participants were from South Africa and India. The median time to TB diagnosis was 4.6 weeks (IQR 2-16.1), with 12 pulmonary and 11 extrapulmonary cases. Differentially expressed miRNA analysis revealed 11 altered miRNAs, with fold change higher than ±2.46 in Cases relative to Controls (P<0.05). Differentially altered metabolite analysis showed no significant alterations in metabolites between Cases and Controls. We found higher TNFα and IP-10/CXCL10 and higher MDC/CCL22 in Controls (p=0.0072). A decision tree algorithm identified gamma-glutamylthreonine and hsa-miR-215-5p as the optimal variables to classify incident TB Cases (AUC 0.965). hsa-miR-215-5p, which targets genes in the TGF-β signaling pathway, was downregulated in Cases. Gamma-glutamylthreonine, a breakdown product of protein catabolism, was less abundant in Cases. Integration of cytokine markers did not improve the AUC.

Conclusion: Use of a machine learning approach in the multi-omics data from advanced HIV participants revealed two variables with the ability to accurately discriminate TB Cases from Controls.
560 OUTCOMES OF ISONIAZID PREVENTIVE THERAPY AMONG CHILDREN LIVING WITH HIV IN KENYA

Dickens O. Onyango¹, Courtney M. Yuen², Samuel Guronion³, Jerphason O. Mecha⁴, Daniel Matemor⁵, Elizabeth Oele¹, John Kinuthia⁶, Grace John-Stewart⁷, Sylvia LaCourse⁸

¹Kisumu County Department of Health, Kisumu, Kenya, ²Harvard Medical School, Boston, MA, USA, ³Kenya Medical Research Institute, Nairobi, Kenya, ⁴Kenyatta National Hospital, Nairobi, Kenya, ⁵University of Washington, Seattle, WA, USA

Background: Tuberculosis (TB) is a leading cause of morbidity and mortality among children living with HIV (CLHIV). Isoniazid (INH) preventive therapy (IPT) is effective in reducing TB incidence among CLHIV. However, there are limited data on the IPT cascade in CLHIV including TB status after 24 months.

Methods: We evaluated the IPT cascade among CLHIV newly enrolled in HIV care in eight high-volume HIV clinics in western Kenya. Medical record data was abstracted for CLHIV aged <15 years who were enrolled from September 2015 through July 2019 (with at least 12 months follow up time) using standardized case report forms. We assessed screening for TB symptoms and IPT eligibility, IPT initiation, IPT outcomes at six months, and TB status at 12, 18 and 24 months. TB incidence rate was compared by IPT initiation and completion status. Correlates of IPT non-initiation and non-completion were assessed using age and sex adjusted robust Poisson regression models.

Results: Overall, 856 CLHIV were newly enrolled in HIV care, of whom 98% (n=841) underwent screening for TB symptoms and IPT eligibility. 2.5% (n=21) were diagnosed with active TB; 98% (n=820) of screened CLHIV were eligible for IPT. Median age at IPT initiation after enrollment in HIV care was 3.6 months (IQR=0.5-10.2). Sixty eight percent (n=559) of eligible CLHIV were initiated on IPT, 78% (n=434) of whom completed the 6-months regimen, 3% (n=16) transferred out, 18% (n=98) lost to follow-up, 2% (n=9) discontinued and <1% (n=1) was diagnosed with active TB. Correlates of non-initiation of IPT included age <5 years (aRR=1.22; 95% CI 1.01-1.48) and viral suppression (aRR=0.78; 95% CI 0.63-1.54). Viral non-suppression (aRR=4.21; 95% CI 1.97-8.99) was associated with lower odds of non-completion of IPT among those who initiated. TB incidence was three-fold higher among CLHIV not initiated vs. initiated on IPT (13.0 vs. 4.2 cases per 1000 child years, p=0.003), and among CLHIV who did not complete vs. completed IPT (8.1 vs. 2.6 cases per 1000 child years, p-value=0.05).

Conclusion: While screening for IPT eligibility was high, later components of the IPT cascade (initiation and completion) were suboptimal. TB incidence at 24 months was higher among CLHIV who neither initiated nor completed IPT than those who completed IPT. There is need to strengthen IPT initiation and completion especially in virally non-suppressed CLHIV.

561 RISK STRATIFICATION FOR IDENTIFYING OPTIMAL TREATMENT DURATION IN ALL MDR-TB PATIENTS

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Background: Multidrug-resistant tuberculosis (MDR-TB) requires extensive chemotherapy, with up to 2 years of treatment with toxic drugs. Using pooled individual patient data, we aimed to identify patient phenotypes who require shorter or longer treatment durations to optimize their chances of cure.

Methods: Published observational and experimental studies of MDR-TB treatment (12938 patients) were considered for analysis. Baseline clinical and treatment features, including drug given, duration and six-month culture conversion, were available. Patients who defaulted or were lost to follow up were excluded. Missing data were imputed using multivariate imputation via chained equations. Risk of unfavorable outcome, which included treatment failure and death, was analyzed through multivariate logistic regression models. We developed a baseline model, using baseline clinical predictors, and on treatment models, including additional treatment features predictors. Based on these models, the individual risk scores were computed, and patients were categorized in high (p_unfavorable>50%), medium (15%<p_unfavorable<50%) and low (p_unfavorable<15%) risk categories.

Results: The final dataset included 5869 patients with treatment success, 628 patients with treatment failure, and 1253 patients who died. In the baseline model, HIV status was the most important predictor of TB unfavorable outcome (aOR 2.3; 95%CI [1.7, 3]; p<0.001). Previous treatment with second line drugs, older age, low BMI, smoking, AFB positivity, extrapulmonary involvement, cavitary disease and resistance to pyrazinamide, fluoroquinolones and injectable drugs were all associated with TB unfavorable outcome (p<0.05). In the on treatment models the use of linezolid, levofloxacin and bedaquiline were associated with lower odds of unfavorable outcome, while the use of kanamycin or capreomycin and PAS were associated with higher odds of unfavorable outcome (p<0.05). Six months culture conversion was the most important predictor of treatment success. The proportion of patients without unfavorable outcome over treatment duration was significantly different between low, medium and high risk phenotypes (Figure 1).

Conclusion: We developed a risk stratification algorithm for patients with MDR-TB based on individual participant meta-analysis of a large dataset. Stratified medicine approaches where treatment duration is selected with greater precision for low, medium and high risk patient phenotypes will maximize chances for cure of all MDR-TB patients.

562 FINAL RESULTS OF THE NIX-TB CLINICAL STUDY OF BPaL REGIMEN FOR HIGHLY RESISTANT TB

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Background: Nix-TB was a single arm prospective study of a regimen of bedaquiline (400 mg daily for 2 weeks followed by 200 mg 3 times a week), pretomanid (200 mg per day) and linezolid (1200 mg per day starting dose, with dose modifications allowed after the first month), given orally for 6 months for Extensively Drug-Resistant (XDR) or treatment intolerant or failed Multidrug-Resistant (MDR) tuberculosis (TB). We report here on long-term efficacy and safety from the recently completed 24-month post-treatment followup in all patients.

Methods: We report the study’s secondary endpoint of bacteriologic or clinical failure or relapse at 24 months post-treatment. Peripheral neuropathy associated with linezolid was assessed serially with standard symptoms rated from none (0) to worst (10) with a change from baseline score calculated at end of treatment and 24 months post-treatment. Detailed methods and the primary endpoint at 6 months after completion of therapy have been reported: https://www.nejm.org/doi/full/10.1056/NEJMoa1901814.
**MENINGITIS AND MORTALITY**

**Conclusion:** Results of this simplified, shortened all oral regimen for highly drug resistant TB show sustained high efficacy through 2-year follow-up from end of treatment. Neuropathy from linezolid was common but improved over 24 months of follow-up. A follow-on trial, Zelixii, that investigates the optimal dose and duration of linezolid in the BPaL regimen, has completed enrollment.

563 **VALIDATION OF CLINIC-BASED POINT-OF-CARE TESTING FOR CRYPTOCOCCAL ANTIGEN SCREENING**

**Methods:** We screened non-pregnant adults 18+ years of age seeking voluntary HIV testing at Ithembalabantu clinic in Umlazi Township, South Africa from November 2017 to February 2019. Participants testing HIV+ and consenting to participate were enrolled, completed a demographic survey and clinical assessment, and received a clinic-based rapid CD4 test. Among those with CD4 count ≤200 cells/mm³, a trained nurse conducted clinic-based CrAg LFA screening (Immy Diagnostics, Norman, USA) and collected a blood sample for standard laboratory-based enzyme immunosassay (EIA) CrAg testing at the BARC lab (Johannesburg, South Africa). Diagnostic performance was assessed by calculating sensitivity, specificity, and positive and negative predictive values compared to standard serum CrAg EIA.

**Results:** Among 1,493 eligible participants screened, 720 (48.2%) tested HIV+ and received rapid CD4 testing. Of the 164 participants with CD4 count ≤200 cells/mm³, all received clinic-based CrAg LFA testing and 162 had gold-standard CrAg EIA testing. CrAg prevalence was 6.1% by serum CrAg LFA and 4.9% by serum CrAg EIA. Serum CrAg LFA testing correctly identified seven of eight CrAg positives by EIA (87.5%, 95% confidence interval [CI]: 50.8-99.9%); specificity was 98.1% (CI: 94.2-99.6%) with three false positives and one false negative. The positive predictive value was 70.0% (CI: 39.2-89.7%) and negative predictive value was 99.3% (CI: 96.0-100.0%) (Table). Diagnostic performance was similar among participants with a CD4 count <100 cells/mm³.

**Conclusion:** Serum CrAg LFA delivered at the point-of-care showed high diagnostic accuracy compared to the gold-standard CrAg EIA, though with a lower positive predictive value suggesting the need for confirmatory testing. These results indicate that serum CrAg LFA may be feasible and accurate to perform at the clinical point-of-care to more rapidly identify HIV+ patients with cryptococcal antigenemia and intervene to prevent meningitis and mortality.

### Table: Diagnostic performance of clinic-based serum CrAg (EIA) against gold standard, laboratory-based serum CrAg EIA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count ≤200 cells/mm³</td>
<td>98.1% (CI: 94.2-99.6%)</td>
<td>87.5% (CI: 50.8-99.9%)</td>
<td>70.0% (CI: 39.2-89.7%)</td>
<td>99.3% (CI: 96.0-100.0%)</td>
<td>39.5% (CI: 9.6-100.0%)</td>
<td>95.3% (CI: 89.5-98.7%)</td>
</tr>
</tbody>
</table>

**564 POINT-OF-CARE CRYPTOCOCCAL ANTIGEN SCREENING FOR PREVENTING MENINGITIS AND MORTALITY**

**Methods:** We conducted a prospective pre-post intervention study of adults presenting for HIV testing in Umlazi Township, South Africa. Participants were enrolled during three phases of CrAg testing — CrAg testing ordered by a clinician (“Clinician-directed testing”, 2013-2015); routine lab-based CrAg reflex testing for blood samples with CD4 <100 cells/mm³ (“Lab reflex testing”; 2015-2017), and a clinic-based intervention with POCT CD4 testing and POCT CrAg testing among persons with CD4 ≤200 cells/mm³, in addition to background standard of care routine reflex testing among those with CD4 <100 cells/mm³ (“Clinic-based testing”; 2017-2019). The laboratory and clinics teams performed serum CrAg by enzyme immunosassay and lateral flow assay (Immy Diagnostics, Norman, USA). We followed participants for up to 14 months to assess the outcomes of ART and fluconazole treatment initiation, cryptococcal meningitis, hospitalization and mortality by baseline CrAg positivity.

**Results:** 3,105 (39.4%) of 7,877 people screened were HIV-positive, of whom 908 (28.7%) had CD4 ≤200 cells/mm³, and were included in analyses. Compared to clinician-directed testing, lab reflex and clinic-based testing increased CrAg screening (p<0.001) and diagnosis of Cryptococcus (CrAg+) (p=0.020). Compared to clinician-directed testing, clinic-based CrAg testing increased the number of participants diagnosed with cryptococcal meningitis (p=0.038). Comparing clinic-based testing to lab reflex testing, there was no significant difference in the cumulative incidence of cryptococcal meningitis (4.5% compared to 4.1%; p=0.829) or mortality (8.1% compared to 9.9%; p=0.463).

**Conclusion:** Among ambulatory adults recently diagnosed with HIV in South Africa, lab reflex and clinic-based CrAg testing facilitated diagnosis of HIV-associated cryptococcosis and fluconazole initiation, compared to clinician-directed CrAg testing. Clinic-based CrAg testing increased the number diagnosed with cryptococcal meningitis, but did not alter hospitalization or mortality rates. In this non-randomized cohort, clinical outcomes were between lab reflex testing and clinic-based point-of-care CrAg testing.
565 CRYPTOCOCCAL MENINGITIS AND CLINICAL OUTCOMES IN PERSONS WITH HIV ACROSS THE GLOBE

Anna Person1, Brenda Crabtree Ramirez2, Ahra Kim3, Fernanda Maruri4, Gilles Wandel1, Richard Moore1, Darma Imran5, Kinh Van Nguyen2, Elizabeth Nalitaya6, Winnie Myunidike2, Bryan Shepherd7, Catherine McGowan, for the iDEA

Background: Cryptococcal meningitis (CM) is a major cause of morbidity and mortality in persons with HIV (PWH). Few studies have examined CM in PWH after expanded global implementation of ART.

Methods: This retrospective cohort study investigated CM incidence and all-cause mortality after CM diagnosis among PWH ≥16 years of age enrolled in an iDEA cohort from 1996-2017. Incidence and incidence rate ratios (IRR) for CM diagnosis were estimated by IeDEA region (North America [NA], Asia-Pacific [AP], Latin America [LA], East Africa [EA], and Southern Africa [SA]), sex, calendar year, time-updated CD4 count, and time-updated ART status using multivariable Poisson regression. Hazard ratios (HRs) for mortality after CM diagnosis were examined using Kaplan-Meier and multivariable Cox regression.

Results: Among 819,641 PWH followed a median of 2.8 years from clinic enrollment, 3961 (0.5%) were diagnosed with CM (incidence 1.15 per 1000 person-years). Incidence over follow-up period varied across regions: 0.89 (95% CI 0.69-1.16) in NA, 0.58 (95% CI 0.41-0.82) in AP, 2.17 (95% CI 1.34-3.52) in LA, 2.08 (95% CI 1.77-2.44) in EA, and 0.42 (95% CI 0.30-0.60) in SA. Unadjusted incidence decreased by calendar year: 1.46/1000py (95% CI 0.46-4.61) in 2000 vs. 0.62 (95% CI 0.34-1.11) in 2015. Current ART use (IRR 0.33, 95% CI 0.25-0.44) and higher CD4 (IRR 3.82 comparing 200 vs. 350 cells/mm3) were associated with lower risks of CM. Among 3961 diagnosed with CM, 1401 were diagnosed before ART start and 2560 (65%) were diagnosed after ART start, with a median time of 255 days from ART start to CM diagnosis (IQR: 56–1051). CM cases were more common among men compared to women (IRR 1.19, 95% CI 1.03-1.36). CM incidence was higher in NA compared to AP, 1.46/1000py (95% CI 0.49-0.73) were associated with higher risks of mortality. Final multivariable model included age (HR=0.94, 95% CI 0.92-0.97), sex (HR=1.11, 95% CI 1.05-1.18), region (HR=1.16, 95% CI 1.10-1.22), and time from ART initiation (HR=1.19, 95% CI 1.15-1.23) for 2-way vs. control.

Conclusion: Few studies have investigated CM in PWH after expanded implementation of ART. Mortality in persons with HIV (PWH) was high, despite increased ART coverage. Significant unexplained variability in CM incidence across regions suggests significant unexplained variation in CM incidence.

566 RCT OF 2-WAY VS 1-WAY SMS MESSAGING TO IMPROVE EFFICACY OF PMTCT-ART IN KENYA

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Kenya Medical Research Institute, Nairobi, Kenya, 2University of Washington, Seattle, WA, USA, 3Kenya Medical Research Institute, Nairobi, Kenya

Background: Prevention of mother-to-child HIV transmission (PMTCT) requires sustained maternal retention and viral suppression. We hypothesized that integrating HIV messages within a maternal child health (MCH) short messaging system (SMS) platform could improve retention and viral suppression.

Methods: The Mobile WACXN 3-arm unblinded randomized trial (RCT) compared 2-way and 1-way SMS messaging to no SMS control. We adapted an existing MCH SMS platform to incorporate antiretroviral therapy (ART) adherence messages based on the Health Belief Model and Social Cognitive Theory. ART and MCH SMS were sent weekly; visit reminders were sent 3 days before appointments. Participants at 6 MCH clinics in Nairobi and Western Kenya were enrolled in pregnancy, randomized, and followed until 24 months postpartum. Clinic attendance, viral load (VL), and infant HIV test results were abstracted from clinic records. Primary outcomes were viral non-suppression (VL ≥1000 c/ml) and on-time clinic attendance (within 2 weeks) after follow-up (≥6 months out of care), and infant HIV-free survival. Intent-to-treat analyses compared arms using generalized estimating equations and Cox regression, adjusted for baseline differences.

Results: Overall 824 pregnant women were randomized (271 2-way, 276 1-way, 277 control). Median age was 27 years (Interquartile Range [IQR] 23-31), gestational age was 24.3 weeks (IQR 18.3-29.6), and time since ART initiation was 1.00 year (IQR 0.02-3.21). During follow-up to 24 months postpartum, 9.78% of 3150 VL assessments were unexpressed. There were no differences in frequency of non-suppression in 1-way vs. control (adjusted Risk Ratio [aRR] 1.02 [95% Confidence Interval [CI] 0.67-1.54]) or 2-way vs. control (aRR 0.80 [95% CI 0.52-1.23]) (Table 1). Overall, 88.9% (95% CI 76.5-95.7%) of visits were on-time, with no difference in 1-way vs. control (aRR 1.00 [95% CI 0.96-1.03]) or 2-way vs. control (aRR 1.01 [95% CI 0.99-1.04]). Incidence of infant HIV acquisition or death during follow-up was 3.01/1000pgy, with no difference in 1-way vs. control or 2-way vs. control arms; overall HIV transmission risk was 0.94%.

Conclusion: In this multi-site RCT in Kenyan PMTCT programs, MCT was rare despite appreciable maternal viral non-suppression. Integrated HIV/MCH messaging did not improve clinic attendance or viral suppression. Potential reasons for lack of effect include high baseline clinic attendance or low impact of generic adherence message. Tailoring SMS to real-time VL results may enhance relevance.

Table 1

| Overall (Median) | Control (n=277) | One-way (n=276) | Two-way (n=271) | Two-way vs control
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<tr>
<td>Viral non-suppression (VL ≥ 1000 c/ml)</td>
<td>14.0% (95% CI 10.0-18.0)</td>
<td>16.0% (95% CI 11.0-21.0)</td>
<td>12.4% (95% CI 8.4-16.4)</td>
<td>0.68 (95% CI 0.49-0.95)</td>
</tr>
<tr>
<td>Two-way vs control (aRR)</td>
<td>1.00 (95% CI 0.64-1.54)</td>
<td>0.89 (95% CI 0.53-1.51)</td>
<td>0.90 (95% CI 0.50-1.62)</td>
<td>0.80 (95% CI 0.52-1.23)</td>
</tr>
<tr>
<td>VL ≥ 1000 c/ml at any time</td>
<td>1237 (31%)</td>
<td>1237 (31%)</td>
<td>1237 (31%)</td>
<td>1.00 (95% CI 0.67-1.54)</td>
</tr>
<tr>
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<td>0.80 (95% CI 0.52-1.23)</td>
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Trendy attendance

<table>
<thead>
<tr>
<th>Proportion of scheduled visits attended within 2 weeks</th>
<th>99%</th>
<th>99%</th>
<th>99%</th>
<th>99%</th>
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<tbody>
<tr>
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<td>0.80 (95% CI 0.52-1.23)</td>
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567 IMPACT OF MODIFIED STANDARD-OF-CARE FOR VIROLOGIC FAILURE IN ART-EXPERIENCED WOMEN

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1University of Zimbabwe, Harare, Zimbabwe, 2The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 3University of Michigan, Ann Arbor, MI, USA, 4Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

Background: Targeting sustained HIV viral suppression, we assessed the combined effectiveness of point-of-care viral load monitoring plus motivational adherence counselling (POCVL+MAC) compared with routine care in ART-experienced peripartum women identified to be at risk of virologic failure in the PROMOTE study in Zimbabwe.
**Methods:** Women with last viral load ≥200 copies/ml and/or pill count outside 90-110% of expected were considered at risk of virologic failure. After tracing, consenting women were randomized 1:1 to continue lab-based viral load monitoring and routine adherence counseling (control) or receive POCVL+MAC from trained primary care nurses and counsellors. Viral load was measured at 0, 3, 6 and 12 months post enrolment. Viral suppression <200 copies/ml at 6 months (primary outcome) was compared between arms through Chi-square testing, and associated factors sought by logistic regression with a 95% confidence interval (CI).

**Results:** Of 448 women screened from December 2018 to July 2019, 157 met the risk criteria: 119 by pill count alone, 38 by last viral load (11/38 also by pill count). 50 women were enrolled (25 control, 25 POCVL+MAC); mean (sd) age was 33 (6) years and 49/50 (98%) were on NNRTI-based ART for an average (sd) duration of 3 (1) years. Baseline sociodemographic characteristics were comparable across arms. At entry, 30 women (60%) had viral suppression; 68% control, 52% POCVL+MAC (Chi2 1.33, p=0.248). At 6 months, only 28 of the 44 retained had viral suppression (64%); 16/21 (76%) control, 12/23 (52%) POCVL+MAC (Chi2 2.74, p=0.098). More POCVL+MAC than control women completed all scheduled counselling and testing sessions; 7/12 (58%) POCVL+MAC, 1/9 (11%) control. Control group women were more likely to be virally suppressed at month 6 (OR 2.93, 95% CI 0.80-10.71). Higher education OR 5.00 (CI 0.56-45.02), pill count averaging 100% OR 1.03 (CI 0.97-1.08), more than 60 minutes travel time to clinic OR 1.75 (CI 0.34-8.98) and HIV disclosure OR 1.59 (CI 0.14-17.56) were associated with an unsuppressed viral load. Only 2 (14%) had treatment switch at or by 6 months, one in each arm.

**Conclusion:** Sustained viral suppression remained elusive in ART-experienced peripartum women identified at risk of viral failure after a 6-month enhanced adherence program. Despite full intervention delivery, POCVL+MAC did not significantly improve viral suppression. There is need to explore other causes of viral un-suppression such as drug resistance, and improve on treatment switch strategies.

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**568 MATERNAL VL TESTING IN BREASTFEEDING FOR TARGETED INFANT HIV TESTING: A SIMULATION**

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**Background:** Challenges encountered by women in the postpartum period contribute to poor ART adherence leading to elevated maternal viral load (VL). In high-burden settings where at least 12m breastfeeding is recommended, this raises concerns about ongoing MTCT, enhanced VL monitoring during breastfeeding is widely discussed but there is little understanding of how detection of maternal viraemia is associated with infant HIV transmission risk.

**Methods:** We used an established Monte Carlo simulation of maternal VL dynamics and MTCT during pregnancy and breastfeeding, calibrated using best available data. Maternal VL was simulated in cohorts of 50,000 women from conception through 2 years postpartum, with a breastfeeding duration of at least 12m postpartum. We used this simulation to estimate, by infant age and maternal VL over time, the ability of maternal VL values to predict cumulative risk of infant HIV transmission. Results are expressed as positive and negative predictive values (PPV and NPV) and positive and negative likelihood ratios (LR+ and LR-). Sensitivity analyses varied distributions of maternal ART use prior to conception and levels of maternal viremia, and incorporated hypothetical information on previous infant HIV testing at birth and 6 weeks of age.

**Results:** In the base simulation, 50% of women were on ART prior to conception and 50% initiated during pregnancy at median 20 weeks gestation. 89% of women had VL<50 cps/ml at delivery, 6% of women never achieved VL<50 cps/ml through 2 years postpartum, and 21% of women experienced VL>1000 cps/ml postpartum after prior VL<50 cps/ml. Cumulative MTCT risks were 2.0% at birth, 3.5% at 6w and 4.4% at 12m postpartum. Breastfeeding maternal VL>1000 cps/ml had low PPVs (5%-25%) in predicting cumulative infant HIV infection, while NPV were >96% in all scenarios. LR+ ranged from 2.5 to 12.5 and were stable across infant ages, with higher values observed in scenarios where an infant was known to have a negative HIV test result at birth (Figure). LR- were lowest (<0.3) for infants with HIV- birth testing but increased over time. Analogous results were observed when using VL thresholds of 10,000 cps/ml and findings were robust to variation in key parameters.

**Conclusion:** Elevated maternal VL observed during breastfeeding becomes more useful as a predictive tool for targeted infant testing when combined with information about prior infant HIV negative test results.

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**569 ANTIRETROVIRAL THERAPY ADHERENCE DURING & POST BREASTFEEDING USING TFV LEVELS IN HAIR**

Teacher G. Nematzadeh1, Pamela Murnane2, Peter Bacchetti2, Hideaki Okochi2, Regina Tallierco1, Alexander Louie3, Yongai M. Chanaiwa4, Tichaona Vhembo4, Mercy T. Mutambanengwe-Jacob1, Tsungai Chipato1, Vongai M. Chanaiwa4,隐山孝明2, Peter Bacchetti2, Landon Myer1, Tichaona Vhembo4, Anthony Bacchetti2, and for the PROMISE Study Team

1University of Cape Town, Cape Town, South Africa, 2Columbia University Medical Center, New York, NY, USA, 3University Health Network, Toronto, Canada, 4Harvard Medical School, Boston, MA, USA, 5United Nations Children’s Fund, New York, NY, USA, 6University of California San Francisco, San Francisco, CA, USA, 7Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 8World Health Organization, Geneva, Switzerland

**Background:** The period post breastfeeding (BF) cessation is vulnerable to attrition and declining adherence to antiretroviral treatment (ART) among women with HIV. Antiretroviral concentrations in hair reflect cumulative exposure over weeks to months. We assessed if ART adherence declined among postpartum women after prior infant HIV negative test results (Figure). POCVL+MAC, 1/9 (11%) control. Control group women were more likely to be virally suppressed at month 6 (OR 2.93, 95% CI 0.80-10.71). Higher education OR 5.00 (CI 0.56-45.02), pill count averaging 100% OR 1.03 (CI 0.97-1.08), more than 60 minutes travel time to clinic OR 1.75 (CI 0.34-8.98) and HIV disclosure OR 1.59 (CI 0.14-17.56) were associated with an unsuppressed viral load. Only 2 (14%) had treatment switch at or by 6 months, one in each arm. Results: In the base simulation, 50% of women were on ART prior to conception and 50% initiated during pregnancy at median 20 weeks gestation. 89% of women had VL<50 cps/ml at delivery, 6% of women never achieved VL<50 cps/ml through 2 years postpartum, and 21% of women experienced VL>1000 cps/ml postpartum after prior VL<50 cps/ml. Cumulative MTCT risks were 2.0% at birth, 3.5% at 6w and 4.4% at 12m postpartum. Breastfeeding maternal VL>1000 cps/ml had low PPVs (5%-25%) in predicting cumulative infant HIV infection, while NPV were >96% in all scenarios. LR+ ranged from 2.5 to 12.5 and were stable across infant ages, with higher values observed in scenarios where an infant was known to have a negative HIV test result at birth (Figure). LR- were lowest (<0.3) for infants with HIV- birth testing but increased over time. Analogous results were observed when using VL thresholds of 10,000 cps/ml and findings were robust to variation in key parameters. Conclusion: Elevated maternal VL observed during breastfeeding becomes more useful as a predictive tool for targeted infant testing when combined with information about prior infant HIV negative test results.
was over, using hair tenofovir (TFV) levels as an objective metric of medication consumption. Moreover, we estimated the association between hair TFV levels and viremia. **Methods:** A subset of women randomized in the PROMISE Study to take ART while BF and continue ART post BF cessation participated in the Hair Substudy in Zimbabwe. Hair and viral loads were collected longitudinally throughout follow-up. Hair TFV levels were measured via validated methods in samples collected after >45 days of TFV-containing ART. We estimated the impact of BF cessation on hair TFV levels via mixed linear models adjusted for demographics, prior viremia and timing of ART initiation. Also, we estimated the relative risk of viremia (>400 copies/mL) associated with each doubling of hair TFV level. **Results:** Of the 55 women included in this analysis (age 19–41), 93% were asymptomatic (WHO Stage I). Hair TFV results (n=305) were available a median of 9 visits/woman from 3–29 months postpartum (up to 1 year post BF cessation). TFV levels were highly variable over time (Figure; median 0.04 ng/mL, range undetected-0.25 across all samples). In adjusted analyses, we observed a non-significant decline in TFV levels after delivery (-1%/month, 95%CI -4,1). TFV levels were significantly higher (95%CI 1-55; p=0.04) post BF cessation than during BF, coupled with a further non-significant 1% monthly decline in TFV levels (95%CI -2.4,14/55 (25%) women were ever viremic postpartum, reaching a median of 15,564 copies/mL (range: 571–81,562). High TFV levels were strongly protective of viremia: the relative risk of viremia per doubling of TFV was 0.43 (95% CI 0.27-0.68, p<0.0001). **Conclusion:** Leveraging an objective metric of ART use during BF and after cessation, we did not observe declining adherence associated with BF cessation. ART adherence is challenging postpartum, and BF cessation may be an opportune time to reinforce adherence support. Varying TFV levels over time and across women throughout the postpartum period highlight the importance of differentiated care for women needing additional support throughout these life transitions to achieve sustained viral suppression and eliminate pediatric HIV.

NRTI-SPARING STRATEGY TO PREVENT PERINATAL HIV TRANSMISSION, ANRS 168 MONOGEST STUDY

Laurent Mandelbrot 1, Roland Tubiana 1, Pierre-Henri Frange 1, Véronique Avettand-Fenoël 1, Gilles Peytavin 1, Ana Canestri 1, Philippe Morlat 1, Cécile Brunet 1, Jeanne Sibiude 1, Delphine Peretti 1, Véronique Chambrin 1, Amélie Chabrol 1, Eida Bui 1, Lucie Marchand 1, Josiane Warszawski 2

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**Background:** Control of HIV viral load (VL) is effective prevention of perinatal transmission. Antiretrovirals (ART) include nucleoside reverse transcriptase inhibitors (NRTIs), but these can have side effects in the fetus. We evaluated a strategy with no NRTI during pregnancy, intrapartum and post-partum prophylaxis in women at low risk of HIV perinatal transmission. Our objective was to estimate, in women switching to darunavir/ritonavir monotherapy (DRV/r) early in pregnancy, the proportion maintaining a VL <50 copies/mL at delivery, with no need of treatment reintensification. **Methods:** A one-arm, open-label, phase 2 clinical trial was performed in 24 French centers. Women with virological suppression and CD4 ≥ 250 cells/µL were enrolled to treatment simplification with DRV/r (600/100 mg bid) in the 1st trimester of pregnancy. Plasma VL was monitored monthly and ART was reintensified in case of viral rebound (>50 copies/mL). Tolerance issues were managed per usual guidelines. Neonates received prophylaxis with nevirapine qd for 14 days. The trial was designed to investigate the virological success rate, with a 2-sided alpha=5% for the exact test comparing the observed proportion of VL<50 copies/mL on monotherapy against a minimum success rate set at p0=85%, and an expected success rate of p1=95%, requiring 80 evaluable patients. **Results:** Of 91 women enrolled, 89 switched to DRV/r monotherapy; 83 were evaluable, 4 miscarried before 22 weeks’ gestation, and 2 changed because of elevated liver enzymes. Treatment was reintensified with NRTIs for viral rebound in not 6/83 (median VL 193 copies/mL; range 78–252), including 2 patients whose rebound occurred on triple ART after screening but before switching to DRV/r. Another 2 patients with VL missing at delivery were considered as failures in the primary per-protocol analysis, resulting in a success rate of 75/83, 90.4% (95%CI, 81.9–95.7%), not significantly above p0=85% (p=0.22). The 2 patients with missing delivery VL had undetectable values on DRV/r until 33 days and 13 days before delivery. If considering them as successes, success was 77/83 = 92.8% (95%CI, 84.9–97.3%), which was significantly higher than 85% (p=0.045). In an intent-to-treat analysis, all 89 women who switched to DRV/r monotherapy had their last VL before delivery <50 copies/mL. There was no case of perinatal HIV transmission.

**Conclusion:** This pilot study suggests that a NRTI-free strategy with careful viral load surveillance can maintain viral suppression during pregnancy.

Table. Virological outcomes in ANRS 168 MONOGEST, n=89

<table>
<thead>
<tr>
<th>Description</th>
<th>H22 WG (n=88)</th>
<th>H22WG (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage outcome</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>DRV/r monotherapy changed before delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>90.6</td>
<td>82.7-95.8</td>
</tr>
<tr>
<td>Changed for inefficacy</td>
<td>7.1</td>
<td>(6)</td>
</tr>
<tr>
<td>Changed for intolerance</td>
<td>2.4</td>
<td>(3)</td>
</tr>
<tr>
<td>VL at pregnancy outcome (4 to 7 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 copies/mL</td>
<td>96.5</td>
<td>10.0–99.3</td>
</tr>
<tr>
<td>&gt;50 copies/mL</td>
<td>0.0</td>
<td>(0)</td>
</tr>
<tr>
<td>Unknown at delivery (4 to 7 days)</td>
<td>3.5</td>
<td>(7)</td>
</tr>
<tr>
<td>Last VL at or before delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 copies/mL</td>
<td>100.0</td>
<td>95.8–1</td>
</tr>
</tbody>
</table>

Primary endpoint among 82 evaluable participants

**Success** | 90.6 | 81.9-95.7 | (75) |
**Failure (including 2 missing VL)** | 9.4 | (6) |
**with missing VL at delivery or success** | 72.2 | 84.9-97.3 | (77) |
**Failure** | 3.5 | (6) |

*For consent withdrawn at 28 weeks with last VL available at 34 weeks, 2 with last VL at 13 and 33 days before delivery (both <50 copies/mL). **Last viral load available at 34 weeks, 52 days before delivery.

MATERNAL WEIGHT AND ADVERSE PREGNANCY OUTCOMES AMONG WOMEN ON ART CONCEPTION

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**Background:** Antiretrovirals such as dolutegravir (DTG) and tenofovir alafenamide (TAF) have been associated with excessive weight gain, but the impact of weight and weight gain on pregnancy outcomes is poorly described among women on antiretroviral treatment (ART). **Methods:** Using data from the Tsepamo birth outcomes surveillance study in Botswana, we evaluated the relationship between maternal weight (and weight gain) and severe birth outcomes (very preterm delivery <32 weeks, very small gestational age <3rd percentile, perinatal death), macrosomia (birthweight >4000g) and maternal hypertension. We estimated the relative risk of each outcome by baseline weight (<24 weeks) and second trimester average weekly weight gain (kg/week from 12 +/-2 to 24 +/-2 weeks). Also, we estimated the relative risk of each outcome by baseline weight (<24 weeks) and second trimester average weekly weight gain (kg/week from 12 +/-2 to 24 +/-2 weeks) using log binomial regression and evaluated effect modification by ART regimen (DTG vs. Efavirenz [EFV]).

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Results: Of 22,828 women on ART at conception with singleton deliveries between August 2014-April 2020, 16,300 (71.4%) had a documented weight measured at <24 weeks gestation (baseline weight) and 4437 (19.2%) had documented weight measured both at 12 (+/-2) weeks and 24 (+/-2) weeks, allowing gestational weight gain calculation. Compared to women with baseline weight 60-70kg, low baseline weight (<50kg) was associated with increased risk of very preterm delivery (aRR 1.30, 95% CI 1.08,1.65) and very small for gestational age (aRR 1.96, 95% CI 1.69,2.28). High baseline weight (>90kg) was associated with increased risk of macrosomia (aRR 3.24, 95% CI 2.16,4.44) and maternal hypertension (aRR 1.79, 95% CI 1.62,1.97) (Figure 1). Baseline weight was not associated with perinatal death. For all outcomes, gestational weight gain showed weaker associations than did baseline weight. Duration of pre-pregnancy ART (years) was associated with higher baseline pregnancy weight for DTG but not for EFV, and the risk of maternal hypertension by baseline weight category was higher for DTG than EFV for all strata.

Conclusion: ART regimens associated with weight gain may reduce the percentage of low weight women at risk for certain severe adverse pregnancy outcomes but increase the number at risk of maternal hypertension. Further research is needed to determine whether weight-based ART treatment strategies could improve maternal and child health.

# 572 PREDICTED LONG-TERM ADVERSE BIRTH AND CHILD HEALTH OUTCOMES IN THE ADVANCE TRIAL

Evangelia E. Baxevanidi1, Sumbul Asif2, Ambar Qavi1, Andrew Hill1, Francois Venter1, Fairlie Lee2, Masebale Masenya2, Gelicia M. Serenata1, Simiso Sokhela1, Nomathemba Chandiwana1

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2University of Leeds, Leeds, UK
3University of Liverpool, Liverpool, UK
4Ezethwa University of the Witwatersrand, Johannesburg, South Africa
5Wits RHI, University of the Witwatersrand, Johannesburg, South Africa

Background: First-line treatment with the integrase inhibitor dolutegravir (DTG) is associated with significant and progressive weight gain, especially among black women and if combined with TAF/FTC. If women become clinically obese after long-term treatment, this could increase risks of adverse birth and child health outcomes. This analysis assessed long-term risks of adverse outcomes in pregnancy and child health from treatment-associated clinical obesity among pregnant women, using data from the ADVANCE trial.

Methods: In the ADVANCE trial treatment-naive patients were randomised to TAF/FTC+DTG, TDF/FTC+DTG or TDF/FTC/EFV for 144 weeks. A systematic review analysed the association between pre-pregnancy obesity and adverse maternal and infant outcomes. The association between pre-pregnancy obesity and adverse outcomes in child health (ages 6-16) was also measured. Risk ratios using the Mantel-Haenszel test with random-effects were calculated. Risk ratios of the pregnancy and child health outcomes were combined with treatment-associated obesity rates from ADVANCE to predict the number of women, infants and children who could experience adverse events in each arm at Week 144.

Results: After 144 weeks of treatment in the ADVANCE trial, the percentage of women with normal baseline BMI becoming clinically obese was 19% for TAF/ FTC+DTG, 5% for TDF/FTC+DTG, and 0% for TDF/FTC/EFV. From baseline to Week 144, the predicted increase of adverse maternal outcomes was 15% with TAF/ FTC+DTG versus 4% with TDF/FTC+DTG, whereas risk predictions for adverse infant outcomes were 12% and 3% in these two treatment groups. Similarly, the predicted risk of adverse outcomes in child health was 28% and 7% on TAF/ FTC+DTG and TDF/FTC+DTG, respectively. No additional adverse events were predicted for pregnant women treated with TDF/FTC/EFV.

Conclusion: Pre-conception weight gain on antiretrovirals could substantially increase adverse outcomes in pregnancy and child health. There are consistent associations between pre-pregnancy clinical obesity and higher risks of adverse maternal, infant and child health outcomes. For every 100 women becoming pregnant after three years of TAF/FTC+DTG treatment, this analysis predicted 18 additional adverse outcomes. New stopping rules may be required to switch women off TAF/FTC+DTG and similar combination treatments, to lessen these risks.

Table 2: Predicted risk of adverse outcomes in pregnancy and child health among 100 pregnant women in the ADVANCE trial receiving ART for 144 weeks.

<table>
<thead>
<tr>
<th>Adverse outcomes</th>
<th>Baseline</th>
<th>Year 3</th>
<th>Absolute change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational hypertension</td>
<td>2.0</td>
<td>4.3</td>
<td>+1.3</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>1.6</td>
<td>2.7</td>
<td>+1.1</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>2.5</td>
<td>3.0</td>
<td>+0.5</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>11.2</td>
<td>11.7</td>
<td>+0.5</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>21.3</td>
<td>20.9</td>
<td>-0.4</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>3.1</td>
<td>5.8</td>
<td>+2.7</td>
</tr>
<tr>
<td>Child overweight/obesity</td>
<td>7.2</td>
<td>11.6</td>
<td>+4.4</td>
</tr>
<tr>
<td>Child small for gestational age</td>
<td>15.4</td>
<td>16.1</td>
<td>+0.7</td>
</tr>
<tr>
<td>Child development disorders</td>
<td>0.9</td>
<td>1.0</td>
<td>+0.1</td>
</tr>
</tbody>
</table>

573 ANTIRETROVIRAL THERAPY CLASS AND GESTATIONAL WEIGHT GAIN: RESULTS FROM PHACS SMARTT

Jennifer Jao1, Carly Broadwell1, Paige L. Williams1, Ellen G. Chadwick1, Lisa Haddad1, Denise Jacobson2, Kathleen M. Powis3, Lynn M. Yee1, Deborah Kanacak4

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4Population Council, New York, NY, USA
5Massachusetts General Hospital, Boston, MA, USA

Background: Excessive gestational weight gain (GWG) is associated with poor maternal, perinatal, and metabolic outcomes. In non-pregnant persons living with HIV (PLHIV), integrase inhibitors (INSTIs) are associated with greater weight gain compared to protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Methods: We evaluated the association of INSTI-based ART with GWG in pregnant PLHIV. The Surveillance Monitoring for ART Toxicities (SMARTT) study has enrolled pregnant PLHIV across 22 US sites since 2007. We analyzed GWG in pregnant PLHIV in SMARTT with available weight data, singleton gestations, and ART (consisting of ≥3 antiretrovirals) initiation at <16 wks) gestational age (GA) from 2007-2019. GWG (delivery weight) - (pre-pregnancy weight or weight taken <12 wks GA) was classified as excessive, adequate, or inadequate using National Academy of Medicine standards. ART was categorized as INSTI-based, PI-based, NNRTI-based, nucleoside reverse transcriptase (NRTI)-based, or ART consisting of ≥3 antiretrovirals (ARV) classes which may have included an INSTI. Modified Poisson models were fit with generalized estimating equations to assess the association of earliest ART class with prevalence of excessive (vs. adequate/inadequate) GWG. Percentages with ART use at conception (ART-C)
and those with ART initiation in pregnancy (ART-I) were analyzed separately. Sensitivity analyses were performed with GWG modeled as excessive vs. adequate GWG.

Results: 1477 pregnancies (847 ART-C, 630 ART-I) of 1282 PLHIV were included. The prevalence of excessive, adequate, and inadequate GWG was 652 (44%), 350 (24%), and 475 (32%) respectively. Age mean (29.3 years), earliest CD4 count (11% <200 cells/mm³) and viral load (60% < 400 copies/mL) in pregnancy, pre-gestational diabetes 3%), and GA at delivery (median 36 weeks) were similar among GWG groups. No associations between ART class and excessive GWG were observed in ART-C or ART-I pregnancies after adjusting for age, race, ethnicity, income, earliest pregnancy viral load, and alcohol, tobacco, or substance use in pregnancy (Table). Results from sensitivity analyses were similar.

Conclusion: Initiation of InSTI-based ART prior to conception or before 16 wks GA was not associated with excessive GWG in pregnant PLHIV in the US. Future studies are needed to assess whether specific InSTIs or switching to an InSTI in pregnancy is associated with GWG.

### Table: Cardiometabolic Health Indicators at 24-28 Weeks Gestation among 231 Pregnant Women in Cape Town, South Africa, Overall and by HIV Status

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Overall</th>
<th>HIV-Uninfected</th>
<th>HIV-Infected</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median CD4</td>
<td>530 (500-570)</td>
<td>545 (510-580)</td>
<td>490 (460-520)</td>
<td>0.02</td>
</tr>
<tr>
<td>ART use</td>
<td>84%</td>
<td>87%</td>
<td>79%</td>
<td>0.05</td>
</tr>
<tr>
<td>Initiation of ART</td>
<td>Median 15 weeks (IQR 11, 20)</td>
<td>15 weeks (IQR 10, 20)</td>
<td>15 weeks (IQR 12, 20)</td>
<td>0.80</td>
</tr>
<tr>
<td>Pregnancy care</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>1.00</td>
</tr>
<tr>
<td>Diagnosed and treated for hypertension</td>
<td>5%</td>
<td>6%</td>
<td>4%</td>
<td>0.50</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>1.00</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1.00</td>
</tr>
</tbody>
</table>

### 575 TIMING OF ART INITIATION IS ASSOCIATED WITH HYPERTENSIVE DISORDERS OF PREGNANCY

Ellen G. Chadwick1, Denise Jacobson1, Lisa Haddad2, Jennifer Jao3, Kathleen M. Pnows4, Deborah Kakanc5, Rebecca Zash6, Alexandra DiPern6, Lynn M. Yee7, for the Pediatric HIV/AIDS Cohort Study

1Ann & Robert H Lurie Children’s Hospital of Chicago, Chicago, IL, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Population Council, New York, NY, USA, 4Northwestern University, Chicago, IL, USA, 5Massachusetts General Hospital, Boston, MA, USA, 6Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA

**Background:** Contemporary cohorts of women living with HIV (WLHIV) appear to be at higher risk of development of hypertensive disorders of pregnancy (HDP) than those in the pre-antiretroviral therapy (ART) era. Whether due to rapid immune reconstitution with specific antiretroviral (ARV) classes when initiated during pregnancy, the ARVs themselves, or other risk factors is unclear. We examined the association of timing of ART initiation and ARV class with risk of new-onset HDP in pregnant WLHIV.

**Methods:** Data were abstracted from medical records of all pregnant WLHIV enrolled in the US-based multisite Surveillance Monitoring for ART Toxicities (SMARTT) study between 1/30/15 and 03/25/19. Pregnancies with multiple fetuses or no ART use were excluded. Hypertension (HTN) categories were classified by clinician diagnoses of HDP and/or timing of anti-HTN medications during pregnancy. New-onset HDP was defined as gestational HTN, preclampsia (PE), or HELLP syndrome (hemolysis, elevated liver enzymes, low platelets); individuals with chronic HTN were excluded from analysis of new-onset HDP. We examined the association of three exposures, each in a separate model, with relative risk of new-onset HDP compared to no HTN by fitting unadjusted and adjusted log-binomial regression models using generalized estimating equations to account for correlations within women with multiple singleton pregnancies. Exposures of interest were: timing of initial ART (preconception, <20 or ≥20 weeks’ gestation) and ART class (PI, NNRTI, InSTI) at conception and initiated during pregnancy.

**Results:** 973/1038 pregnancies were singleton with complete data on HTN diagnosis; 948/973 WLHIV received ART during pregnancy (median age 28.9 years; 69% identified as Black, 25% as White, 26% as Hispanic ethnicity.) Overall, 9% of pregnancies had new-onset HDP, 10% had chronic HTN and 81% had no HTN. Relative risk of new-onset HDP did not differ by ART class, but those initiating ART after 20 weeks had a significantly increased risk of HDP (adjusted RR 1.94 [95% CI 1.13-3.32] compared with those on ART at conception (see Table).

**Conclusion:** In this large, diverse cohort of WLHIV, 9% of pregnancies had new-onset HDP, with ART initiation at >20 weeks of gestation conferring higher risk, suggesting a potential role for rapid immune reconstitution later in pregnancy. However, in contrast to prior hypotheses, HDP was not associated with ART class.
The risk of gestational hypertension with use of dolutegravir at conception

Rebecca Zash1, Ellen Caniglia2, Gloria Mayondo3, Modiegis Diksie1, Judith Mabuta1, Denise Jacobson3, Katherine Johnson1, Mompati Mmalane4, Joseph Makheka1, Shahnin Lockman2, Roger Shapiro1

1Beth Israel Deaconess Medical Center, Boston, MA, USA, 2New York University Langone Medical Center, New York, NY, USA, 3Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 4Harvard TH Chan School of Public Health, Boston, MA, USA, 5University of Massachusetts, Worcester, MA, USA, 6Brigham and Women’s Hospital, Boston, MA, USA

Background: Hypertension (HTN) in pregnancy is an important cause of adverse maternal and fetal outcomes. Dolutegravir (DTG) has been associated with excess weight gain and adverse cardiometabolic effects in non-pregnant adults, which could lead to increased HTN during pregnancy. We therefore compared the risk of HTN in pregnancy among women on DTG to women on efavirenz (EFV), and to women without HIV (WWoH).

Methods: Tsepamo birth surveillance study data was used to emulate a hypothetical (target) trial where women would have been randomized to DTG vs. EFV-based ART pre-conception and followed to delivery along with a control group of women without HIV. The main outcome was gestational HTN, defined as the onset of HTN (SBP >140 or DBP >90) at any gestational age (GA), excluding those with chronic HTN. Secondary outcomes included women with chronic HTN and 1) any HTN (SBP >140 or DBP >90 at any GA), 2) severe HTN (SBP >160 or DBP >110 at any GA) and 3) early HTN (onset of hypertension before 34 weeks GA). We fit multivariable log-binomial regression models to estimate the RR of each hypertensive outcome.

Results: Of 176,321 deliveries between Aug 2014-Oct 2020, 16,412 fit criteria for the target trial, including 2079 women conceiving on DTG, 3735 women conceiving on EFV and 10,598 WWH. Women in the DTG and EFV groups were less likely to be married than WWH (7.6% vs. 9.3% vs. 18.8%), while women on DTG and WWH were more likely to be nulliparous than women on EFV (15.0% vs. 12.1% vs. 9.2%). Gestational HTN occurred in 12.8% of women on DTG, 10.1% of women on EFV, and 14.7% among WWH. Compared with DTG, the risk of gestational HTN was lower with EFV (aRR 0.79, 95% CI 0.68,0.93) and higher among WWH (1.17, 95% CI 1.03,1.33). Any HTN, severe HTN and early HTN were also less common among EFV-conception and more common among women without HIV than DTG-conception (Table).

Conclusion: Gestational HTN is more common among women on DTG at conception than women on EFV at conception, but less common than in WWH. Further research is needed to determine if this is related to maternal weight.

576 THE RISK OF GESTATIONAL HYPERTENSION WITH USE OF Dolutegravir AT CONCEPTION

577 TRAJECTORIES OF PERINATAL DEPRESSION SYMPTOMS IN KENYAN WOMEN LIVING WITH HIV

Osborn Lusi1, Anna Larsen2, Keshet Ronen3, Barbra A. Richardson3, Wenwen Jiang4, Bhavna Chohan4, Daniel Mateimo1, Jennifer A. Unger3, Alison L. Drake2, John Kinuthia5, Grace John-Stewart2

1Kenya Medical Research Institute, Nairobi, Kenya, 2University of Washington, Seattle, WA, USA, 3Kenya Medical Research Institute, Nairobi, Kenya

Background: Pregnant and postpartum (PP) women living with HIV (WLWH) experience elevated risk of depression. We examined longitudinal patterns and predictors of depressive symptoms among perinatal Kenyan WLWH who participated in a randomized trial of mHealth interventions.

Methods: We followed 824 WLWH enrolled in pregnancy through 24 months postpartum (PP) in 6 Nairobi and Nyanza region maternal child health (MCH) clinics. Study nurses assessed depressive symptoms during pregnancy, 6 weeks, 6, 12, 18, and 24 months PP using Patient Health Questionnaire-9 (PHQ-9). Psychosocial factors were evaluated using the: Stigma Scale for Chronic Illness, Medical Outcomes Study Social Support Survey, Abuse Assessment Screen for intimate partner violence (IPV), and Household Food Insecurity Access Scale. We evaluated prevalence of moderate-to-severe depressive symptoms (MDD; PHQ-9 score ≥10) and pre-defined symptom trajectories (no MDD ever, MDD in pregnancy only, PP MDD only, and MDD in pregnancy and PP). We identified correlates of any PP MDD using generalized estimating equations with Poisson link and independent correlation clustered by participant.

Results: Of 824 WLWH who participated in a randomized trial of mHealth interventions. Methods: We followed 824 WLWH enrolled in pregnancy through 24 months postpartum (PP) in 6 Nairobi and Nyanza region maternal child health (MCH) clinics. Study nurses assessed depressive symptoms during pregnancy, 6 weeks, 6, 12, 18, and 24 months PP using Patient Health Questionnaire-9 (PHQ-9). Psychosocial factors were evaluated using the: Stigma Scale for Chronic Illness, Medical Outcomes Study Social Support Survey, Abuse Assessment Screen for intimate partner violence (IPV), and Household Food Insecurity Access Scale. We evaluated prevalence of moderate-to-severe depressive symptoms (MDD; PHQ-9 score ≥10) and pre-defined symptom trajectories (no MDD ever, MDD in pregnancy only, PP MDD only, and MDD in pregnancy and PP). We identified correlates of any PP MDD using generalized estimating equations with Poisson link and independent correlation clustered by participant.

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578 ANTENATAL PROGESTERONE PROTECTS AGAINST FETAL GROWTH RESTRICTION IN WOMEN WITH HIV

Madelyn Conner1, Bellington Vwalia1, Stephen Cole1, Bethany Freeman1, Chileshe Mabula-Bwalya1, Marc Peterson1, Poujo Saha1, Elizabeth Stringer1, Margaret P. Kasaro1, Jeff Stringer1, Joan T. Price1

1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 2University of Zambia, Lusaka, Zambia, 3University of North Carolina in Zambia, Lusaka, Zambia

Background: Widespread use of antiretroviral therapy (ART) has substantially reduced vertical HIV transmission. Second-line ART regimens that include protease inhibitors (PI) have been linked to adverse pregnancy outcomes such as maternal preeclampsia (PEC) and fetal growth restriction. Progesterone supplementation reverses PI-associated placental angiogenic changes in mouse models.

Methods: The Improving Pregnancy Outcomes with Progesterone (IPOP) trial was a phase III double-masked study that randomized pregnant women with HIV in Lusaka, Zambia to weekly injections of either 17 alpha-hydroxyprogesterone caproate (17P) or placebo starting at 16-24 weeks gestation. The trial’s primary outcome, preterm birth (<37 weeks or stillbirth at any gestational age), did not differ by randomization group. In a secondary analysis, we evaluated the risk of PEC and small gestational age (<3%) (SGA3) associated with PI-based ART and evaluated whether 17P modified this risk.

Results: From Feb 2018 to Jan 2020, 800 pregnant women with HIV were randomized to either 17P (399) or placebo (401), of whom 24 (3%) were on a PI-based regimen; 11 randomized to 17P and 13 to placebo. Overall, 75 (9%) delivered an SGA3 infant, 5/24 (21%) on PI vs. 70/762 (9%) on non-PI regimens (RR 2.3; 95% CI 1.0, 5.1). Risk of SGA3 was 28/392 (7%) among those randomized to 17P compared to 47/394 (12%) randomized to placebo (RR 0.6; 95% CI 0.4, 0.9). In the placebo group, risk of SGA3 was higher among those on PI (4/13; 31%) compared to those on other regimens (43/381; 11%) (RR 2.8; 1.2, 6.6), while the risk was similar in the 17P group (7.1% on PI vs. 9.0% non-PI) (RR 1.3; 0.2, 8.6). There were 15 (2%) preeclampsia events, 2/24 (8%) on PI vs. 13/776 (2%) on non-PI regimens (RR 5.0; 95% CI 1.2, 21.1). Risk of PEC was similar in 17P (9/401; 2%) compared to placebo groups (6/399; 2%) overall, although women randomized to placebo on PIs (2/13; 15%) compared to other regimens (7/388; 2%) had higher risk of PEC (RR 8.5; 95% CI 2.0, 37.1), while among those receiving placebo 0/11 on PIs compared to 6/388 (2%) of those on other regimens developed PEC.

Conclusion: Protease inhibitor use was associated with PEC and SGA. 17P was protective against SGA3 overall and may have modified the risk of both SGA3 and PEC in those with PI exposure. These findings support that progesterone may protect against placental vascular changes and the resultant adverse outcomes associated with PI-based therapy.

579 VAGINAL MICROBIOME AND INFLAMMATION PREDICT PRETERM BIRTH IN ZAMBIAN WOMEN WITH HIV

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Background: Maternal HIV increases the risk of spontaneous preterm birth (sPTB). A Lactobacillus-deficient, anerobe-dominant vaginal microbiome has been associated with sPTB, but few studies have assessed this association in the setting of HIV.

Methods: We performed a nested case-control study in a cohort of HIV-infected and uninfected women in Zambia who experienced either sPTB (case) or term birth (control). Vaginal swabs collected between 16-20 gestational weeks were used for whole metagenome sequencing of the vaginal microbiome and assays of 12 inflammatory markers. Assays were repeated at 24-36 weeks in the HIV-infected women. We used VIRGO, a non-redundant gene catalogues of the vaginal microbiome to group samples into 7 metagenomic community state types (mgCST) and created a vaginal inflammatory score for each corresponding sample with factor analysis.

Results: Of 221 participants, 29 (13%) had sPTB and 192 (87%) delivered at term. Median Shannon diversity index (SD) was highest in the 41 (19%) HIV-infected women with detectable plasma viral load (1.13, IQR: 0.85–1.66; p<0.001) and the 44 (20%) with undetectable virus (1.17, IQR: 0.51–1.66; p=0.01) vs. the 136 (62%) without HIV (0.74, IQR 0.35–1.26). Inflammatory scores were positively correlated with SD (coefficient +0.66, 95% CI 0.28, 1.03; p=0.001), and highest among the anaerobe-dominant mgCST2–mgCST6 (Fig 1A). HIV was associated with predominance of Gardnerella and mixed anaerobes in mgCST5 (17% vs. 6%; p=0.02) and mgCST6 (27% vs. 11%; p=0.002), and a lower prevalence of L. crispatus-dominant mgCST7 (4% vs. 23%; p=0.001) (Fig 1B). Relative abundance of Gardnerella above 1% (PR 2.8; 95% CI 1.4, 5.6), L. iners above 26% (PR 2.4; 95% CI 1.21, 4.78), and L. crispatus below 0.02% (PR 4.9; 95% CI 1.08, 17.9) were each associated with sPTB. Vaginal inflammation at baseline (APR 2.8; 95% CI 1.5, 5.2) and, in the HIV-infected women, an increase in SD at repeat sampling (APR 2.5; 95% CI 1.1, 5.6) were associated with sPTB.

Conclusion: HIV in pregnancy is associated with a diverse, anaerobe-dominant vaginal microbiome, which in turn correlates with inflammation. Gardnerella and L. iners species predicted sPTB while L. crispatus, rare in women with HIV, was protective. These findings suggest the risk of PTB faced by women with HIV may be mediated by the vaginal microbial and inflammatory environment and could be a target for novel preventive therapies.
580 PREGNANCY RATES & CLINICAL OUTCOME COMPARISONS AMONG WOMEN LIVING WITH HIV: HPTN 052

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Background: Early studies on the natural history of HIV infection in women suggest that untreated HIV infection is associated with reduction in fertility. HPTN 052 was one of few interventional clinical trials that allowed participation of pregnant women. We explored pregnancy and health characteristics in this cohort of women living with HIV.

Methods: HPTN 052 was a multi-country randomized trial evaluating the effect of early cART on sexual transmission of HIV among serodiscordant couples which allowed participation of pregnant women although contraception was recommended. Data on all HIV-infected index female participants was combined as a single longitudinal cohort. Cox proportional hazards models were used to infer associations between hypothesized static and time varying predictors and time to first pregnancy after study enrollment (Table). Analyses used SAS version 9.4.

Results: 869 women were followed for an average of 5.7 years (SD = 1.6). 115 women were pregnant at enrollment; 196 women had 1, 60 had 2 and 15 had > 3 pregnancies after enrollment; 532 women were never pregnant during the study. The mean time on ART was 4.61 years (SE = 0.09) for women ever pregnant (WEP) vs 4.67 years (SE=0.09) for women never pregnant (WNP). Annual pregnancy rate was 7.3% (362/4962 PY). AIDS was observed in 9.5% of WEP (32/337) vs 12.8% of WNP (68/532), p = 0.17; STIs were present in 24% of WEP (65/275) vs. 30.6% of WNP (68/222), p = 0.08. In the multivariable model, CD4 cell count increases were associated with decreased pregnancy (adj HR=0.89 (0.84, 0.96)), but not virus load suppression (adj HR=1.01 (0.74, 1.38)). Younger women and those in countries with lower contraceptive coverage had higher pregnancy rates. Partner seroconversion (as a surrogate for unprotected sex) was associated with higher pregnancy rates in univariate but not multivariable model; parity was associated with lower pregnancy rates in the univariate but not multivariable model; self-reported condom use was not associated with pregnancy (Table). Women on PI-based ART had higher pregnancy rates, likely due to study treatment guidelines.

Conclusion: Clinical outcomes were similar between ever pregnant/ never pregnant women. CD4 cell increase over time and parity were associated with reduced pregnancy suggesting that access to cART and contraceptives empowered women living with HIV in making family planning decisions.

Table: Predictors of Pregnancy Among HIV+ Women following enrollment into HPTN 052. Results from Cox Proportional Hazards models.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count (per 100 cells/μL, increase)</td>
<td>0.82 (0.87, 0.86)</td>
<td>0.89 (0.84, 0.96)</td>
</tr>
<tr>
<td>Contraception use at last sex (past 3 mos), yes</td>
<td>1.07 (0.75, 1.52)</td>
<td>1.08 (0.73, 1.54)</td>
</tr>
<tr>
<td>Baseline ART regimen, no ART vs. initiated ART, yes</td>
<td>1.15 (0.51, 2.60)</td>
<td>0.88 (0.26, 2.91)</td>
</tr>
<tr>
<td>PI-based regimen</td>
<td>2.19 (1.72, 2.79)</td>
<td>2.25 (1.74, 2.91)</td>
</tr>
<tr>
<td>HIV RNA &lt;= 400 copies/mL, yes</td>
<td>0.79 (0.61, 1.03)</td>
<td>1.01 (0.74, 1.38)</td>
</tr>
<tr>
<td>Contraceptive coverage, yes</td>
<td>0.29 (0.20, 0.42)</td>
<td>0.28 (0.15, 0.42)</td>
</tr>
<tr>
<td>Parity (ref. = 0)</td>
<td>2x</td>
<td>0.72 (0.53, 0.97)</td>
</tr>
<tr>
<td>Age (cot. ref. = 18-24)</td>
<td>25-34</td>
<td>0.60 (0.44, 0.82)</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>0.14 (0.09, 0.22)</td>
<td>0.13 (0.08, 0.21)</td>
</tr>
<tr>
<td>Partner seroconversion during study, yes</td>
<td>2.62 (1.32, 4.77)</td>
<td>1.56 (1.00, 2.43)</td>
</tr>
</tbody>
</table>

581 INCREASED C-SECTIONS AND PRETERM BIRTHS IN SARS-CoV-2 INFECTION DURING PREGNANCY

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Background: SARS-CoV2 infection severity during pregnancy and possible consequences for exposed newborns information is still unknown. The objective of this study is to analyse clinical and epidemiological characteristics of a SARS-CoV2 infected women during pregnancy and their newborns cohort.

Methods: Multicentric observational study from the Spanish GENE-COVID cohort (participating in RECLIP). Infected pregnant women and their newborns born from 15 March to 31 July with a 15 days follow up were included. Data regarding epidemiological, clinical, virological and immunological characteristics of the patients was collected.

Results: Globally, 105 pregnant women with a median age of 34 (IQR: 29 – 37) years old and 107 newborns were included in the study. Median gestational age at diagnosis was 36.9 (IQR: 33.4-39.2) weeks, and 6.7% as women were diagnosed in the second trimester. More than 34% of the women presented at least one comorbidity and almost 65% of women had COVID19 symptoms and 43% of them were treated for the infection. Overall, 30.8% had COVID19 pneumonia and 4.8% were admitted to the intensive care unit (ICU) needing invasive mechanical ventilation. The rate of positive RT-PCR at delivery was 61.9%. There was a 36.2% rate of caesarean sections, associated with pneumonia during pregnancy OR:4.2 (95% CI 1.5,12.0) and lower gestational age at delivery OR:0.7 (95% CI 0.6,0.9). Regarding newborns, 46.7% were male, 66.4% breastfed, with median Apgar 1’ of 9 and Apgar 5’ of 10. Almost 6% were small for gestational age and 16.8% needed admission to the neonatal ICU. Oxygen was needed by 12.1% and surfactant by 5.6% newborns. Prematurity rate was 20.6%, associated with pneumonia during gestation OR:7.0 (95% CI: 2.23,2.8) and with a positive RT-PCR at delivery OR:6.5 (95% CI 1.8,31.8). No associations were found with age, comorbidities or blood group. No vertical transmission was reported but one newborn was horizontally infected. Two newborns died, one due to premature causes and another of unexpected sudden death during early skin-to-sk in contact after delivery.

Conclusion: Even there is no vertical transmission reported in this cohort, we found a case of horizontal transmission. SARS-CoV2 infection could produce COVID19 pneumonia during pregnancy, that increases caesarean sections and prematurity rates worsening exposed newborns prognosis.

582 PREGNANCY OUTCOMES OF WOMEN POSITIVE FOR SARS-CoV-2 COMPARED TO WOMEN TESTED NEGATIVE

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1Assistance Publique–Hôpitaux de Paris, Paris, France, 2Université de Paris, PARIS, France, 3Université Paris-Saclay, Orsay, France

Background: Potential effects of infection with SARS-CoV-2 in pregnant women are still conflicted. Initial symptoms for COVID-19 are often unspecific, it is thus clinically relevant to know if a positive naso-pharyngeal real time reverse transcription PCR (RT-PCR) for SARS-CoV-2 at evaluation is predictive of perinatal outcomes. Our objective was to determine the impact of SARS-CoV-2 infection among women presenting with symptoms indicating a virological test.

Methods: We conducted a retrospective study including all pregnant women tested for SARS-CoV-2 by RT-PCR in respiratory tract samples from March 12-May 1st in two tertiary referral obstetric units in the Paris metropolitan area. Indication for tests were one or more of the following symptoms: fever (>38°C), coughing, dyspea, anosmia, myalgia, rhinorrhea, nausea or vomiting or diarrhea. Clinical and biological characteristics at initial evaluation and perinatal outcomes were compared with student Chi2 or Fisher tests as appropriate.

Results: 123 patients were tested for SARS-CoV-2, 35 were positive (28%). Pregnancy outcomes were available for 93% (N=114). Mean gestational
age at testing was similar between the groups (29.2 vs 30.1WG, p=0.53). The symptoms which were more frequent in women with positive PCR were anosmia: 22.2% (12/55) vs 9% (6/68), p=0.05, and myalgia: 33% vs 17%, p=0.04. Concerning biological characteristics, women with positive PCR were more often of blood type A (vs type 0; p=0.004), more often lymphopenic (47% vs 5%, p<0.001), there was a trend towards more abnormal APTT ratio (>1:2) (p=0.07). Hospitalization rates were higher for women tested positive: 41.8% vs 21.3%, p=0.02. All 8 women hospitalized in intensive unit care were tested positive. Preterm birth (<37WG) was higher in the group of women tested positive (30.2 vs 13.3%, p=0.029) and there was a similar trend for severe preterm (<32WG) birth (15% vs 5.0%, p=0.07). Among the 78 women not delivered 15 days after the test, the rate of preterm birth was similar in both groups: 17.1% (6/35) vs 11.6% (5/43), p=0.47. We found no difference in the rate of preeclampsia (4% vs 5%) or post-partum hemorrhage (15.1% vs 9.8%, p=0.41). Birthweight Z-score did not differ between the groups (-0.3 vs -0.1, p=0.32).

Conclusion: In a symptomatic pregnant population tested positive for SARS-CoV-2 compared to negative patients with similar characteristics, COVID-19 infection seemed to increase medically indicated preterm births, especially during the 15 days after the RT-PCR result.

583 COMPLICATED SARS-CoV-2 NEUTRALIZING ANTIBODY RESPONSE IN CORD BLOOD VERSUS MOTHERS

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Background: Maternal antibodies are important for infant immunity, and understanding the maternal and umbilical cord antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection will be important for neonatal management and maternal vaccination strategies.

Methods: The dynamics of maternal/umbilical cord antibody responses to the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein were analyzed in 81 samples from 69 pregnant women studied between April 2020 and January 2021. Binding IgG, IgA and IgM antibodies to RBD were measured by enzyme-linked immunosorbent assay (ELISA) in both maternal and cord blood.

Neutralization was assessed using codon-optimized full-length G614 Spike enzyme-linked immunosorbent assay (ELISA) in both maternal and cord blood. Neutralization rates were significantly lower and did not show positive titers for IgA and IgM. Among the samples tested, 71.4% had neutralization titers. Interestingly, the neutralization capacity of plasma from cord blood was negative when compared to maternal blood (mean titer of 20 vs 2128 respectively), suggesting that cord blood does not have capacity to neutralize the SARS-CoV-2 virus.

Conclusion: In this cohort study, maternal IgG, IgA and IgM antibodies to RBD of SARS-CoV-2 were seen in maternal samples. However the cord blood IgG levels were significantly lower and did not show positive titers for IgA and IgM. Although both maternal and cord blood has RBD binding antibodies, there is no neutralization seen in any of the cord blood tested compared to respective maternal blood. Our findings demonstrate that maternally-derived SARS-CoV-2 specific antibodies lack neutralization potential to provide neonatal protection from COVID-19.
586 PREVENTING VERTICAL HIV TRANSMISSION IN THE UK: SUCCESSES AND EMERGING CHALLENGES

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Background: The UK has met 90-90-90 targets since 2017 and a major success has been the low vertical HIV transmission rate (VTR), declining from 2.1% in 2000-01 to 0.28% in 2015-16 among diagnosed women living with HIV (WLHIV). The British HIV Association (BHIVA) recommends follow-up of all infants of WLHIV at 18-24mths (18-24Ab), regardless of negative PCR testing, to establish infection status and formula feeding to eliminate postnatal transmission risk. BHIVA guidelines state that virologically suppressed women on antiretroviral therapy (ART) with good adherence who choose to breastfeed may be clinically supported to do so.

Methods: The Integrated Screening Outcomes Surveillance Service (ISOSS), part of Public Health England's Infectious Diseases in Pregnancy Screening (IDPS) Programme, monitors all pregnancies to diagnosed WLHIV and their infants in the UK. All children diagnosed with HIV <16yrs are reported to ISOSS, with enhanced data collection for those vertically infected in the UK. Clinical Expert Review Panels establish circumstances surrounding transmissions and contributing factors. We describe maternal characteristics and VTRs among singleton liveborn infants in 2017-18 with HIV status reported by 30/09/20 and cases of supported breastfeeding since 2012.

Results: There were 1527 livebirths, with 89% (1353/1520) of mothers of WLHIV and their infants in the UK. All children diagnosed with HIV <16yrs are reported to ISOSS, with enhanced data collection for those vertically infected in the UK. Clinical Expert Review Panels establish circumstances surrounding transmissions and contributing factors. We describe maternal characteristics and VTRs among singleton liveborn infants in 2017-18 with HIV status reported by 30/09/20 and cases of supported breastfeeding since 2012.

Conclusion: There were 1527 livebirths, with 89% (1353/1520) of mothers of WLHIV and their infants in the UK. All children diagnosed with HIV <16yrs are reported to ISOSS, with enhanced data collection for those vertically infected in the UK. Clinical Expert Review Panels establish circumstances surrounding transmissions and contributing factors. We describe maternal characteristics and VTRs among singleton liveborn infants in 2017-18 with HIV status reported by 30/09/20 and cases of supported breastfeeding since 2012.

Figure: Vertical transmission rates of HIV among diagnosed women in the UK 2000-18

587 PRETERM BIRTH, BREASTFEEDING, ANTENATAL ARV REGIMEN, AND 24-MONTH HIV-FREE SURVIVAL

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Background: PROMISE 1077BF/1077FF was a multi-site, randomized, open-label, perinatal trial comparing the relative safety/efficacy of proven ARV regimens during pregnancy and breastfeeding (BF) among HIV+ women not meeting treatment criteria at trial entry. Previous results found <1% HIV transmission risk but increased risk of adverse pregnancy outcomes including preterm (<37 weeks) delivery for mothers receiving antenatal triple ARVs (ART) compared to zidovudine (ZDV) alone. This raised concerns that antenatal ART and associated PT risk might impact later child survival.

Methods: In period I, pregnant HIV+/HIV- women were randomized 1:1 to ZDV alone or ZDV/3TC/lopinavir-ritonavir (ZDV-ART) while HIV+/HIV+ women were randomized 1:1:1 to ZDV alone, ZDV-ART or tenofovir/emtricitabine/lopinavir-ritonavir (TDF-ART). In period II, enrollees were randomized 1:1:1 to the 3 regimens. We analyzed overall and HIV-free survival at 24-months for liveborn infants, based on (1) gestational age at delivery: PT (<37 weeks) or term (≥37 weeks) and (2) maternal antepartum ART randomization. Kaplan-Meier method was used to calculate survival probabilities and 95% confidence intervals (CIs). Cox proportional hazards regression with BF (with ARVs) as a time-varying covariate was used to calculate hazard ratios (HR) and 95% CIs.

Results: For periods I-II there were no significant differences in 24 month HIV free survival between antenatal ZDV-ART or ZDV-alone regimens, but time-varying BF was associated with decreased risk of HIV infection or death (adjusted HR 0.14 (95%CI: 0.09-0.20)). In period I only, TDF-ART had >2-fold increased risk of HIV or death by 24 months compared to ZDV-ART (HR 2.37, 95%CI: 1.21-4.64); time-varying BF was associated with decreased risk of HIV or death (HR 0.95 (95%CI: 0.93-0.97)). In both periods, among 3482 liveborn infants (51% male, 97% African), there were 64 (2.2%) deaths and 53 (1.8%) HIV infections among 2914 term infants; and 62 (10.9%) deaths and 18 (3.2%) HIV infections among 586 PT infants (Figure). PT birth was associated with decreased 24-month HIV free survival: 0.85 (95%CI: 0.82-0.88) vs term birth: 0.96 (95%CI: 0.95-0.96); and 79% of PT deaths were neonatal (age < 30 days).

Conclusion: In PROMISE, PT birth <37 weeks was significantly associated with lower 24 month HIV-free survival, while BF was associated with increased HIV-free survival. Finding Interventions to decrease risk of PT birth for HIV+ mothers and prevent related infant deaths remain a high priority.
588 TRENDS IN HIV PREVALENCE AMONG PREGNANT WOMEN IN BOTSWANA: AN OPPORTUNITY FOR PrEP?
Andrew Kapoor1, Aamirah Musa2, Modiegi Deseke2, Gloria Mayondi1, Judith Mabuta1, Mompali Mmalane1, Joseph Makhemba1, Chelsea Morroni1, Rebecca Zash1, Roger Shapiro3, for the Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, Liverpool School of Tropical Medicine, Liverpool, UK

Background: Young women in sub-Saharan Africa are at particularly high risk of HIV acquisition. Recent shifts toward ‘test and treat’ strategies have potential to reduce transmission in this age group, but have not been widely studied outside of clinical trials. Using data from nationwide surveillance among pregnant women in Botswana, where a ‘test and treat’ strategy began in May 2016, we describe trends in HIV prevalence over time and highlight the opportunity for targeted prevention.

Methods: The Tsepamo study abstracted data from obstetric records of all women delivering at eight government hospitals in Botswana between 2015–2019, approximately 45% of all births in the country. Maternal HIV status was documented in >99% of records along with other demographic data. We used descriptive stratified analyses to identify prevalence trends by age group and year. We evaluated decreases in HIV prevalence over time using Cochran–Armitage test for linear trend and performed an adjusted analysis using multivariable logistic regression to identify factors associated with decline in HIV prevalence.

Results: Among the 120,755 antenatal records reviewed during the study period, the overall prevalence of HIV infection was 24.1%. Prevalence differed by site of delivery, ranging from 16.1%–28.2% and increased markedly with age (Figure 1). Lower educational attainment (OR=3.28 95% CI 3.07-3.50) and being unmarried (OR=1.98 95% CI 1.88 – 2.08) were associated with HIV infection. Prior pregnancy was a strong risk factor for HIV (OR=2.22 95%CI 2.10-2.29); prevalence was 10.0% with a first pregnancy, 21.0% with a second, and 39.2% with a third or greater. The same age adjusted trends were seen in women aged 15-24, with 2-3-fold increase between a first and third pregnancy (Figure 1). Prevalence decreased linearly during the 5-year study period from 25.8% to 22.7% (p <0.001). Among age specific strata, the greatest absolute decline over the 5-year study period was seen in those ages 25-39, who showed a 5.4% absolute decrease from 2015 to 2019 (Figure 1). Minimal declines were seen in those 15-24, with a decrease of only 1.0% over the same period.

Conclusion: While overall trends in Botswana show HIV prevalence declining among pregnant women, prevalence among the youngest age group has remained stagnant. Preventative intervention utilizing pre-exposure prophylaxis (PrEP) should be prioritized during and immediately after the first pregnancy.

589 CLINIC EXPERIENCES ASSOCIATED WITH HIV OUTCOMES AMONG YOUNG MOTHERS LIVING WITH HIV
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Background: Understanding adolescent motherhood and HIV infection in resource-constrained settings is critical. In this study, we report HIV treatment-related outcomes and clinic factors associated with poor treatment outcomes among adolescent mothers living with HIV in South Africa.

Methods: All adolescent girls living with HIV from 52 clinics and 9 maternity obstetric units in a health district in South Africa were approached (90.1% and 96% enrolled in each facility type), resulting in n=792 young women living with HIV aged 11-25 participating in the study. Self-reported questionnaires—using validated tools where available—were piloted with n=25 HIV-positive adolescents. Participants who had at least one child before the age of 20 were coded as being adolescent mothers. Analyses included two steps: (1) comparing HIV-related outcomes among adolescent mothers living with HIV (n=354) to nulliparous adolescent girls and young women living with HIV (n=438), and (2) identifying clinic-level factors associated with poor HIV-related outcomes for both groups, using STATA16.1.

Results: Socio-demographic characteristics, HIV related outcomes and clinic experiences among participants by motherhood are included in Table 1. Among adolescent mothers living with HIV, n=225 (82.6%) knew their child’s HIV-status and fifteen (5%) had at least one child living with HIV. One in six (16%) adolescent mothers living with HIV was not on consistent ART during pregnancy and breastfeeding, and another third (34%) started after the first trimester. Compared to non-mothers, adolescent mothers living with HIV were more likely to report past-week non-adherence (26.0% vs 18.7%, p=0.014), and past-year treatment interruptions (32.8% vs 18.7%, p<0.001). Adolescent mothers were more likely to report ART unavailability due to clinic stock-outs (19.3% vs 13.9%, p=0.036) and be unsatisfied with how they were treated at health facilities during routine care (13.8% vs 7.8%, p=0.005). In multivariate analyses, ART stockouts were associated with past-week non-adherence (aOR=32.65 95%CI 15.64-68.14, p<0.001) and treatment interruptions (aOR=20.72 95%CI 8.85-48.54, p<0.001). Adolescent-sensitive services were associated with lower odds of treatment interruptions (aOR=0.31 95%CI 0.09-0.97 p=0.044).

Conclusion: More effective and acceptable clinic-based services are critical to treatment adherence and reducing onward HIV-transmission to partners and their HIV-exposed children.
590 LOWER INSULIN SENSITIVITY EARLY IN LIFE WITH IN UTERO HIV/ART EXPOSURE IN BOTSWANA

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Background: Few data exist on early life metabolic perturbations in newborns with in utero HIV and antiretroviral therapy (ART) exposure who are HIV uninfected (HEU).

Methods: We measured pre-prandial insulin and glucose at birth (within 72 hours of life) and 1 month (mo) to calculate Homeostasis Model Assessment for Insulin Resistance (HOMA) in newborns HEU and newborns HIV-unexposed (HU) enrolled in the Tshilo Dikotla study in Botswana from 2016-2019. Data on sociodemographics, family history of diabetes (DM), maternal body mass index (BMI), gestational DM (GDM), HIV disease, and ART history, as well as newborn gestational age (GA), anthropometrics, and feeding were collected. HOMA was log-transformed; 2 scores for birth weight (BWZ) and length (BLZ) were calculated. Linear mixed models were fit to assess the association between in utero HIV/ART exposure and average HOMA from birth to 1 mo of age, adjusting for confounders. Subgroup analyses were performed in newborns HEU to assess the association of in utero ART (tenofovir (TFV), emtricitabine (FTC)/dolutegravir (DTG) vs. TFV/FTC/darunavir (EFV)) with HOMA.

Results: Of 450 newborns, 144 were HUU. Maternal age (30 vs. 24 years, p<0.001) and proportion completing ≥ secondary school education (89% vs. 69%, p<0.001) was higher among women of newborns HEU vs. HUU respectively; family history of DM, maternal BMI, GDM, and tobacco/alcohol/substance use, as well as newborn GA, BWZ, BLZ, and proportions of newborns exclusively breastfed in the first mo of life were similar between groups. Among mothers of newborns HEU, 47% had a CD4 >500 cells/mm, and 93% a viral load (VL) <40 copies/mL at enrollment; 56% were on ART at conception, 59% received TFV/FTC and 4% TFV/EFV. Median birth and 1 mo HOMA was 0.38 vs. 0.30 and 0.76 vs. 0.62 for newborns HEU vs. HUU respectively. Mean log HOMA from birth to 1 mo of age was 0.68 units (p=0.037) higher in newborns HEU vs. HUU after adjusting for family history of DM, maternal age, education, BMI, GDM, and tobacco/alcohol/substance use, as well as newborn sex, preterm birth, and birth anthropometrics. (Table) Among newborns HEU, there was no association between in utero ART and HOMA from birth to 1 mo of age after adjusting for maternal CD4, VL and ART use at conception in addition to the confounders above.

Conclusion: In this cohort, newborns HEU had lower insulin sensitivity compared to those HUU from birth to 1 mo. Future studies to evaluate the long-term significance of this early life metabolic alteration are warranted.

591 IMMUNOLOGIC PREDICTORS OF NEURODEVELOPMENT IN HIV-EXPOSED AND -UNEXPOSED CHILDREN

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Background: Children who are HIV-exposed, uninfected (HEU) have worse neurodevelopmental (ND) outcomes compared to children who are HIV-unexposed (HU), but assessing ND in resource-poor settings is challenging. We measured cognitive, language and motor development in HEU and HU paired with pro- and anti-inflammatory plasma biomarkers in each child to identify potential biomarkers of poor ND outcomes.

Methods: We enrolled 82 Kenyan children (44 HEU and 38 HU) between ages 18-36 months old. 81 plasma biomarkers, including cytokines, chemokines, growth factors and soluble immune checkpoints, were quantified using magnetic bead based multiplex assays. ND was measured using the Bayley Scales of Infant and Toddler Development, 3rd edition. Composite scores for cognition, language, and motor domains were used for the analysis. We computed Spearman’s rank correlations of 81 plasma biomarkers and 6 demographic/social/clinical factors (age, gender, WAMI [water/sanitation/ income], maternal education, prematurity and malnutrition) with cognitive, language and motor scores. To identify predictors of ND, variables with p<0.1 from the Spearman’s correlation test were jointly considered in a multiple linear regression model, and Bayesian model averaging (BMA) was performed to identify a parsimonious subset of those most useful for predicting ND outcomes, with a posterior inclusion probability (PIP) ≥0.5 considered as significant.

Results: The correlation analysis resulted in 2, 6 and 10 variables with p<0.1 for cognitive, language and motor scores respectively to input the BMA for HEU. Fibroblast growth factor-2 (FGF2) predicted language scores (PIP=0.64) and IL-22 predicted motor development (PIP=0.75) in HEU. In HU, the correlation analysis resulted in 16, 15, and 5 variables for cognitive, language and motor scores respectively to input the BMA. Hepatocyte growth factor (HGF) and IL-5 predicted cognitive scores (PIP=0.68 and 0.62) and CXCL13 and IL-7 predicted motor outcomes (PIP=0.59 and 0.51). Language scores in HU were predicted by maternal education (PIP=0.79), IL-1a (PIP=0.67), IL-2R (PIP=0.84), and IL-5 (PIP=0.76).

Conclusion: Immunologic biomarkers predicted ND outcomes more frequently than social, demographic and clinical factors. The predictors of cognitive, motor and language outcomes differed between HEU and HU. Interestingly, IL-22, an inflammatory cytokine was the strongest predictor in HEU while CD25, an anti-inflammatory cytokine was the strongest predictor in HU.

952 GROWTH FALTERING AND DEVELOPMENTAL DELAYS IN HIV-EXPOSED UNINFECTED INFANTS IN UGANDA

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Background: HIV exposed but uninfected infants (HEI) are at increased risk of impaired early linear growth and cognitive development. We examined associations between pre and postnatal growth and subsequent neurodevelopment in Ugandan HEUs, hypothesizing that early insults may explain alterations in both somatic growth and brain development.

Methods: We prospectively followed a cohort of HEUs from birth to 18 months of age. The height, weight, head circumference, and mid-upper arm circumference (MUAC) were collected and compared to the World Health
Organization growth charts. The Malawi Development Assessment Test (MDAT) was performed at 12 and 18 months of age to examine developmental milestones. The Color Object Association Test (COAT) was used at 18 months of age to assess declarative memory. **Results:** 375 marker-child pairs were enrolled. Mothers were median 28 years old and 32% had a known diagnosis of HIV prior to pregnancy. The cohort included HEUs who were female (53%), premature (11%) and had low birth weight (LBW) (7.6%). Follow up was completed at 6 weeks (n=147 HEUs), 12 months (n=109 HEUs), and 18 months (n=170 HEUs) of age. Eight infants tested positive for HIV and were excluded from the study. 197 HEUs were lost to follow up at 18 months of age. The final cohort consisted of 170 HEUs who completed the MDAT at 18 months of age. The number of HEUs stunted (32%, 43%, and 58%) and underweight (7.4%, 15% and 15%) increased at 6 weeks, 12 months and 18 months of age respectively. HEUs had behavioral scores on the MDAT that were similar to the reference children population. The mean score on the COAT was 5.5 compared to 6.9 in the reference children population. The MDAT score at 18 months of age showed cross-sectional correlation with weight (ρ=0.36, p<0.001), height (ρ=0.41, p<0.001), and head circumference (ρ=0.26, p=0.0011), and MUAC for age (ρ=0.34, p=0.0014). Failure to thrive (FTT), defined as crossing two major percentile lines downward on the weight-for-age growth chart, was observed in 21% HEUs during the first 18 months of life. Failure to thrive (FTT) was associated with lower MDAT scores (ρ=0.042) at 18 months of age. Lower weight-for-height z-scores were associated with lower COAT scores (ρ=0.32, p=0.0017). LBW (<2500g) predicted lower MDAT score (ρ=0.0010) at 18 months of age. **Conclusion:** In a prospective cohort of HEUs in Uganda, LBW, stunting, and FTT were common and were associated with lower attainment of developmental milestones and lower declarative memory at 18 months.

**593 MARKERS OF RESILIENCE IN YOUNG ADULTS WITH PERINATAL HIV EXPOSURE OR INFECTION**

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**Background:** Resilience is defined as positive adaptation in the context of risk or adversity. Perinatal HIV exposure (PHEU) or infection (PHIV) can adversely affect youth development, yet few studies have examined resilience in young adults with PHEU (YAPHEU) or PHIV (YAPPHIV). We evaluated factors contributing to resilience, marked by attainment of three milestones in the transition from adolescence to adulthood: high school graduation, postsecondary education, and current employment. **Methods:** In this prospective analysis, prevalence of each milestone was calculated for YAPHEU and YAPPHIV age 19-30 enrolled in the PHACS AMP Up cohort who were previously followed in the PHACS AMP study of children and adolescents. Potential influences included executive function, cognitive efficiency (working memory [WMH] and processing speed [PSI]), behavioral/social-emotional functioning, caregiver mental/physical health, HIV disease markers for YAPPHIV, and total adversity (adverse life events plus cognitive/behavioral/social-emotional risks and caregiver health). The most recent AMP assessment was used for each influence; outcomes were measured at Year 1 in AMP Up. Separate robust Poisson regression models were fit to evaluate associations between each influence and each outcome, adjusting for demographic factors and PHIV status (also considered as an effect modifier). Multiple imputation was used for missing data. **Results:** Participants (N=315; YAPPHIV=228) were 58% female, 67% Black, 27% Hispanic. Compared to YAPHEU, YAPPHIV were older (mean 20.8 vs 20.2 years) and more often from families with higher median income. In adjusted models (Figure), high school graduation and postsecondary education were more likely in those with higher cognitive efficiency, higher parent-reported executive function, and lower total adversity. For high school graduation, age-appropriate behavior (per parent report) was an additional asset. For postsecondary education, additional assets were age-appropriate adaptive skills (parent and child reports) and lack of difficulty in emotional functioning (child report). Current employment was associated with higher cognitive efficiency and, among YAPPHIV, lower nadir CD4. PHIV status did not modify associations. **Conclusion:** Different skill sets affect attainment of academic vs employment milestones. To promote resilience, targeted services addressing cognitive or behavioral challenges in childhood and adolescence may encourage attainment of milestones among young adults affected by HIV.

**594 DISCOVERY OF LARGE CLONES IN CHILDREN CARRYING PROVIRUSES CONSISTING OF A SINGLE LTR**

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**Background:** A prior study revealed that HIV-infected T cell clones arise rapidly in children born with HIV and that clones can persist for at least 9 years on ART. Little is known, however, about the proviral structures within the clones and about their dynamics over time. We investigated the proviral structures and the dynamics of the most expanded T cell clones in early treated children using new approaches to quantify and genetically characterize proviruses within cell clones. **Methods:** Clones were investigated in children with suppressed viremia on ART for >6 years after initiating treatment at a median age of 6.1 months (range: 1.8-9.9). A new integration site specific proviral amplification (ISSPA) assay was designed to characterize the proviruses in large cell clones identified by standard integration site analysis. Primers were used to amplify the full-length HIV from either the 5′ or 3′ proviral-host junction to the human genome and/or from each LTR-host junction. Clones were also quantified by standard integration site analysis. Primers were used to amplify the full-length HIV from either the 5′ or 3′ proviral-host junction to the human genome and/or from each LTR-host junction. Clones were also quantified by standard integration site analysis. Primers were used to amplify the full-length HIV from either the 5′ or 3′ proviral-host junction to the human genome and/or from each LTR-host junction. Clones were also quantified by standard integration site analysis.
approach. ISSAQ is normalized for cellular input and targets the human genome region adjacent to the integration site in the first round of PCR and the unique integration site junction in the second round.

Results: 9 large clones comprising 1-12.5% of all integration sites detected were evaluated in 5 children (Table 1). The provirus in 8 of the 9 clones consisted only of a single, full-lengthLTR. The 9th clone had a LTR with a 240kb deletion spanning the R region. ISSAQ showed a statistically significant change in clone size in 2 of the 9 clones in two different children: one increased in size from 22 to 140 integration events per 1 million cells and the other decreased from 15 to 2. The other 7 infected cell clones remained remarkably stable in size over the 1.5-year sampling interval.

Conclusion: The largest cell clones in children contained highly defective proviruses consisting of a single LTR or partial LTR. Most of these clones were stable in size but a subset showed large changes. Whether early treatment in children enriches for clones with highly defective proviruses or if such clones are more common than previously recognized is unknown. The effects of single LTRs on cellular function should be investigated as they contain promotor sequences.

<table>
<thead>
<tr>
<th>Age at ART initiation (months)</th>
<th>Time to viral suppression (years)</th>
<th>Integron chromosom/orientation</th>
<th>Gene orientation</th>
<th>% of total integrations detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td>C1/C1</td>
<td>Single LTR</td>
<td>5.9%</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>C2/C1</td>
<td>Single LTR</td>
<td>5.9%</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>C2/C2</td>
<td>Single LTR</td>
<td>5.9%</td>
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<tr>
<td>4</td>
<td>4.1</td>
<td>C2/C1</td>
<td>Single LTR</td>
<td>5.9%</td>
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<tr>
<td>5</td>
<td>1.8</td>
<td>C2/C2</td>
<td>Single LTR</td>
<td>5.9%</td>
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Proportional and gene orientation is indicated with ‘+’ referring to forward and ‘-’ to reverse

595 TOTAL HIV DNA LEVELS CORRELATE WITH PLASMA IL-2 LEVELS IN THAI INFANTS BUT NOT ADULTS

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Background: The infant immune response to infection differs from that of adults, with decreased Th1 cytokine production and more rapid HIV disease progression. We measured the production of cytokines in plasma samples from vertically HIV-infected Thai infants at the time of diagnosis and annually after suppressive early antiretroviral therapy (ART).

Methods: Plasma samples from 92 vertically infected Thai infants living with HIV who were enrolled in the HIVNAT209 study were analyzed. Infants initiated ART immediately after diagnosis within the first 6 months of life (median 2 mo.). Samples were collected within 1 week of ART initiation (n=34) and yearly thereafter through 5 years of suppressive ART (n=134 total). Plasma cytokine levels were measured by LumineX. Total HIV DNA levels were measured in blood CD4+ T cells by real-time PCR. Comparisons were made with 42 Thai adults living with HIV who initiated ART in adult acute HIV infection (AHI), HIV exposed uninfected infants (HEU, n=10, median 15 mo. old), and HIV-adults (n=9).

Results: At the time of ART initiation, infants had higher levels of CXCL13, IL-2, and TNFa (p<0.0001), IL-8 and TSLP (p<0.0001), and MIP-1a (p<0.05) than adults with AHI despite the latter having higher viral loads (p<0.001). Levels of CXCL13, IP-10, and MIP-1a were positively correlated with viral load at the time of ART initiation in both adults and infants, whereas IFNγ, IL-2, TSLP, IL-8, sCD40L, and TNFa were correlated with viral load in infants only. Following ART, children maintained higher levels of CXCL13, IL-2, MIP-1a, sCD40L, TNFa, and TSLP (p<0.0001), IL-8 (p<0.001), and IFNγ (p<0.01) than adults. To determine if the elevated cytokine levels are driven by age or HIV status, we compared plasma cytokine levels in HEU and HIV-adults. We found higher levels of sCD40L (p<0.001), CXCL13 and IL-2 (p<0.01), and TSLP (p=0.05) in HEU compared to HIV-adults, suggesting that these cytokines are elevated in infants regardless of HIV status. Interestingly the levels of plasma IL-2 correlated with total HIV DNA levels in children on successful ART (r=0.32, p<0.001), but not in adults (Fig 1).

Conclusion: Infants produced robust cytokines both at diagnosis and after ART, many at higher levels than adults living with HIV. In infants on ART, plasma IL-2 levels correlated with total HIV DNA levels. As IL-2 levels were also elevated in HEU, these data suggest that higher IL-2 levels may drive seeding, proliferation, or survival of latently infected cells, and thus reservoir persistence, in children.

596 FASTER INITIAL VIRAL DECAY IN FEMALE CHILDREN LIVING WITH HIV

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Background: Women living with HIV have lower HIV RNA levels and higher CD4 cell counts than men. However, limited data exist regarding sex bias and viral decay in children with HIV. We investigated the sex differences in viral decay and control of viremia in HIV perinatally infected children who suppressed viral load within 12 months of treatment initiation and describe the association between viral decay and DNA reservoir size.

Methods: We analysed data from 25 patients from four European cohorts of perinatally infected children (CARMA Study). We estimated the breakpoints on viral decay trends to distinguish viremia control phases and slopes using a piecewise regression model. The effect of sex on the viral decay was analysed using a multivariable mixed model regression and cell lifespan was extrapolated using the ushr tool. The association between viral decay in phase-I and DNA reservoir size was inferred using a multivariable Poisson regression model.

Results: Females (n=17, 68%) and males presented similar HIV RNA levels (5.7 [5.25;6.0] vs. 5.7 [5.13;5.81, p=0.883]) and % CD4 (29.0 cells/mm3 [28.0;30.0] vs. 25.0 [23.0;27.0, p=0.29]) at ART initiation. No differences were found between sexes relating age at ART, age at HIV diagnosis, or time to suppression. However, females reached phase-II significantly earlier than male (3.0 months [1.44;4.85] vs. 6.79 months [5.14;9.94], p=0.023). For each month elapsed, females had faster viral decay than male (interaction coefficient= -0.01±0.001).

Conclusion: Females presented faster phase-I viral decay regardless their age at ART initiation, baseline %CD4, or baseline RNA levels.
INCREASED IMMUNE ACTIVATION AND EXHAUSTION IN VERTICALLY HIV-1–INFECTED YOUNG ADULTS

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Background: Vertically HIV-infected children show irreversible immune damage associated with HIV-1 infection and early antiretroviral treatment (ART) exposure. The objective of the study is to assess immune activation and senescence of vertically HIV-infected patients once they reach adulthood compared to non-HIV-infected subjects.

Methods: Vertically HIV-infected group (HIV n=32) under suppressive ART for at least 5 years were selected from the Paediatric AIDS Research Network of Spain (coRISpe) and cryopreserved samples from the Spanish HIV BioBank. HIV group was compared with a non-HIV-infected Healthy Donors group (HD n=28) matched by age and sex. Subset distribution and activation, proliferation, senescence and exhaustion markers on T cells and natural killer (NK) cells was studied on peripheral blood mononuclear cells by multiparametric flow cytometry.

Results: HIV (24 years [IQR:22-28] median age, 12% male, 794 [IQR:599-981] median CD4+ T cells, 198 [IQR:76-330] median CD4+ Nadir, 4 [IQR:1-6] years median age at ART initiation, 20 [IQR:18-23] years since ART initiation and 8 [IQR:7-10] years under virological control) show differences in CD4-T maturation subsets (defined by CD45RA and CD27 expression, Fig A), high HLA-DR/CD38, CD127, TIM-3 and low CD69 expression on CD4-T (Fig B) and CD8-T cell subsets compared with HD. Regarding NK phenotype, HIV showed low frequency of CD56dim (p=0.057), CD16high (p=0.02), high percentage of CD56high NK subsets (p=0.166) and increased levels of HLADR and TIM-3 expression on CD56dim and CD56high subsets compared with HD (Fig C). Focusing on HIV, strong and direct correlations were observed between activation and senescence (HLA-DR CD38 and CD57, Fig D-E) and exhaustion (TIGIT, PD-1, p=0.002; r=-0.76, p=0.013; r=-0.45 respectively) on CD4-T cells with CD4/CD8 Ratio. On NK cells, HLA-DR and CD69 (Fig F-G) NK2D2 and NK2A expression correlated with CD4/CD8 Ratio (p=0.027; r=-0.40; p=0.033; r=0.39 respectively). Direct associations between age at ART initiation and frequency of CD16high NK subset and expression of exhaustion (CD57, TIM-3) and activation (NKP30) markers on NK subsets were also observed (p=0.08; r=0.31; p=0.02; r=0.41; p=0.06; r=0.32 respectively).

Conclusion: Vertical HIV infection leads to an irreversible immune damage not normalized once adulthood is reached, shown by an increased activation and exhaustion levels in adaptive and innate immune components that are associated with clinical parameters including ratio CD4/CD8 and age at ART initiation.

VIRAL-RESERVOIR LANDSCAPE IN EARLY-TREATED VERTICALLY HIV-INFECTED ADOLESCENTS

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Background: Perinatally HIV-1 infected adolescents and young adults (PHIV), who started ART therapy very early in their life with no history of viral failure, represent a unique cohort to characterize the effects of long-term ART on viral reservoir structure and composition.

Methods: 10 PHIV who initiated ART at a mean age of 4 months with durable viral control (plasma HIV-RNA<50 cp/ml) since treatment initiation (median 15 years) were enrolled at Bambino Gesù Children’s Hospital. For comparison, 41 HIV-1 ART-treated adults (ART) with undetectable viral loads for a median of 9 years (range, 2-19) and 64 untreated HIV-1 Elite Controller (ECs) with undetectable viral loads for a median of 9 years (range, 1-24 years) were included in this study. Genomic DNA was extracted from total PBMCs and diluted to single genome levels, followed by near full-length next generation viral sequencing. Overall, a median of 9.3x10^6, 6.6x10^6 and 1.9x10^6 of PHIV, ECs and ART, respectively.

Results: We obtained 317, 1385 and 2388 individual proviral sequences in PHIV, ECs and ART, respectively. The proportion of both intact and defective proviral sequences with hypermutations, internal inversions, premature stop codons and PSI defects was significantly higher in ART and ECs compared to PHIV, while there was no difference in terms of large deletion between the 3 groups (A). Importantly, we found that the median frequency of total (B) and defective (C) HIV-1 DNA sequences in PHIV was significantly lower than in ART-treated adults (p<0.0001), whereas there was no difference between PHIV and EC. Intact proviral sequences were detected in 1 out of 10 PHIV, and their relative frequency was similar between EC and PHIV (0.2 copies/million in ECs vs 0.1 copies/million in PHIV); in contrast, the frequency of intact HIV-1 sequences was significantly lower in PHIV compared to ART-treated adults (0.3 copies/million in PHIV vs 2.1 copies/million in ART, p<0.0001). Notably, all intact sequences detected in PHIV were part of a sequence-identical clone. Interestingly, 1 PHIV, who started ART at 5 months, under treatment for a recorded time of 24 years, showed 19 defective and no intact proviral sequences in more than 80 million PBMCs.

Conclusion: These data suggest that PHIV display a viral reservoir landscape similar to ECs but significantly different from ART-treated adults. Future studies will be necessary to characterize the specific characteristics of early-treated and long-term virally suppressed adolescents.
599 AUTOLOGOUS INFANT ADCC RESPONSES CORRELATE WITH LOWER MTCT AND BETTER OUTCOMES
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Background: Previous studies have shown that neutralizing antibody (nAb) responses do not account for the lack of HIV-1 transmission from infected mothers to their breastfed infants. We hypothesize that antibodies capable of inducing antibody-dependent cellular cytotoxicity (ADCC) associate with decreased mother-to-child-transmission and correlate with improved outcomes in infected infants.

Methods: ADCC responses were assessed using an infection-based luciferase assay in maternal and infant samples against viruses incorporating HIV-1 envelopes isolated from the chronically infected mothers. Human Isotyping Multiplex assays were used to quantify the magnitude of IgG present in the samples. Differences, correlations, and outcomes among transmitting (TM) versus non-transmitting mothers (NTM) and among HIV infected (HI) as compared to HIV exposed uninfected (HEU) infants were assessed using Wilcoxon rank-sum test, Spearman correlation, and Kaplan Meier analysis.

Results: Breastfeeding, Antiretroviral, and Nutrition (BAN) cohort samples were obtained from 13 mother infant dyads around 40 days prior to documented transmission and from 23 pairs without transmission at a similar time after birth. TMs and NTMs had similar plasma virus levels (p=0.63) and absolute CD4 count (p=0.95), and HI and HEU infants had similar birthweight (p=0.34). HEU infants had higher ADCC responses compared to matched HI infants (p=0.05). NTM as compared to TM also had higher plasma ADCC responses although the difference was not statistically significant (p=0.15). HI infants with low ADCC responses (below the median for HI group) had more serious adverse events compared to those with high ADCC responses (hazard ratio, 7.33; 95% confidence interval, 1.17 to 45.94; p=0.03). Infant and maternal ADCC was highly correlated (p=0.53; p=0.001), and the magnitude of the responses decreased in the infants over time (p=0.57; p=0.0003). ADCC and nAb responses were not correlated in the infants (p=0.19; p=0.37) or the mothers (p=0.28; p=0.15). There was no significant difference in the magnitude of IgG between TM and NTM (p=0.54) or HI versus HEU (p=0.21), and IgG levels did not correlate with ADCC activity in maternal (p=0.25) or infant (p=0.48) plasma.

Conclusion: Higher infant ADCC and not nAb responses against strains circulating in their infected mother correlate with both decreased breast milk mother-to-child-transmission and lower infant morbidity. Additionally, the quality not quantity of IgG is important for these ADCC responses.

601 SWITCHING EFAVIRENZ TO RILPIVIRINE IN VIROLOGICALLY SUPPRESSED ADOLESCENTS WITH HIV
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Background: Efavirenz (EFV) based-antiretroviral therapy (ART) is commonly used for first-line treatment in adolescents and children with HIV but is associated with neuropsychiatric and metabolic side effects. Rilpirivine (RPV) has a more favorable tolerability profile and switching EFV to RPV in virologically suppressed adults was safe and efficacious, but data in adolescents are limited.

Methods: Open-label, single-arm, study in adolescents aged 12-18 years old receiving EFV plus NRTIs for >3 months with virologic suppression. Efavirenz was switched to a RPV 25 mg tablet once daily, HIV-1 RNA viral load, CD4 cell counts, fasting total cholesterol, triglyceride, and glucose were assessed over 48 weeks. Neuropsychiatric adverse events, depression and quality of life (QOL) were also evaluated.

.DIAGRAM

600 DOLUTEGRAVIR AND VIRAL LOAD SUPPRESSION AMONG PEDIATRIC PATIENTS IN CARE IN ZAMBIA
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Background: Preliminary results from the ODYSSEY trial led to FDA approval for the use of Dolutegravir (DTG) in treatment-naive or experienced young children.

Methods: We reviewed a cohort of HIV+ individuals aged ≤18 years with recorded HIV care visit between January 1, 2019 and December 31, 2019 in five health facilities in Lusaka, Zambia. Routine programmatic data, including demographic, clinical, and laboratory measures were extracted from electronic medical records. The outcome, viral load non-suppression, (viremic), was defined as a viral load >100 copies/ml. We created a fixed-effects regression model and Kaplan-Meier curves using Stata IC 15.1.

Results: A total of 2245 individuals with a median age of 8 years were included in the analysis. Median time from HIV diagnosis to ART initiation was 14 days (IQR: 0-56 days). ART initiation between 10-14 years is associated with 2.7 (p=0.017) times the odds of being viremic compared to individuals aged 15-18 years. ART regimen was found to be significantly associated with viremia, with those on regimens containing protease inhibitors (PI) having significantly higher odds of viremia compared to those on non-PI/non-DTG regimens (OR: 2.13, p-value: <0.001). Remarkably, DTG-containing regimens were associated with significantly lower odds of viremia compared to those on non-PI/non-DTG regimens (OR=0.15, p<0.001). Survival analysis show significantly lower cumulative incidence estimates of viremia among those on DTG-containing regimens compared to non-DTG/PI-containing regimens at three years of follow-up (log-rank: <0.001) (Fig. 1). Objective assessments of ART adherence were not available in this dataset to distinguish between improved efficacy and tolerability.

Conclusion: DTG-associated regimens were associated with superior viral suppression among children living with HIV in Zambia, supporting their inclusion in the national guidelines for all eligible pediatric clients. The lack of an objective ART adherence tool is a limitation of this study. Improved fidelity of DTG-containing pediatric ART regimens are likely to attain sustained viral suppression and improved health outcomes.

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602 MINDFULNESS TO IMPROVE ART ADHERENCE AND ACCEPTANCE IN YOUTH LIVING WITH HIV: AN RCT
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Background: Individuals 13-24 years old make up an alarming and disproportionate 21% of new HIV diagnoses. Unfortunately, this age group is less engaged in care and only half as likely to achieve HIV viral suppression (seen in only 30%) than older individuals, leading to significant vulnerability to illness and limiting broader efforts to end the HIV epidemic

Methods: Our previous research found that mindfulness programming for HIV-infected youth was promising, showing improved coping, life satisfaction, and potentially decreased HIV viral load. This NIH-funded RCT aimed to further explore the effect of evidence-based mindfulness-based stress reduction (MBSR) vs. health education control (HT) on HIV medication adherence in HIV-infected youth. Data were collected at baseline, 3, 6, and 12 months. Generalized linear additive modeling was conducted to determine differences by arm over time. In-depth interviews were conducted with 20 individuals from both study arms at baseline and follow-up.

Results: Seventy-four 13-24 year old participants from medical clinics at two major academic centers completed baseline data collection and were randomized to MBSR or HT. Following program participation, MBSR participants had greater increases in medication adherence (p=0.001) and greater decline in HIV viral load (p=0.052) at 3-month follow-up, but not at 6 or 12 months. Qualitative data describe challenges of managing HIV as a stigmatized, chronic condition, amidst significant stressors and social inequalities. MBSR participants had greater increases in medication adherence (p=0.001) and greater decline in HIV viral load (p=0.052) at 3-month follow-up, but not at 6 or 12 months.

Conclusion: This mixed-methods RCT finds that MBSR participants had greater increase in self-reported medication adherence and reduction in HIV viral load following program participation, but not at follow-up. Also, MBSR participants perceive greater capacity for acceptance of the complex and difficult thoughts and emotions related to living with HIV, leading to improved medication adherence. Given the significant vulnerability of this population and the importance of achieving higher rates of HIV viral suppression to decrease transmission and end the HIV epidemic, MBSR remains a promising approach to enhance the treatment of HIV-infected youth and young adults.

604 IMPACT 2014 24-WEEK PK AND SAFETY OF DORAVIRINE/3TC/TDF IN ADOLESCENTS WITH HIV-1
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Background: Doravirine (DOR) is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) active against both wild-type HIV-1 and the most common NNRTI-resistant variants. DOR, alone or as a fixed dose combination (FDC) of DOR/lamivudine (3TC)/tenofovir disoproxil fumarate (TDF), is approved for treatment in antiretroviral-naïve and virologically-suppressed adults with HIV-1 infection. IMPAACT 2014 investigated the pharmacokinetics (PK) and safety of DOR as a component of the FDC in adolescents with HIV-1 through 24 weeks. The 100mg dose of DOR evaluated in this study was previously confirmed based on 2-week safety and single dose PK in adolescents ≥45kg.

Methods: Adolescents with HIV-1, between the ages of 12 and 18 years and weighing at least 45 kg who were antiretroviral therapy (ART)-naïve or virologically-suppressed on stable ART, were enrolled into an open-label trial evaluating the once daily FDC tablet regimen of DOR 100mg, 3TC 300mg, and TDF 300mg. Safety, virologic and PK data were evaluated through Week 24 of therapy.

Results: Forty-five adolescents (43 virologically-suppressed on stable ART and 2 ART-naïve at enrollment) were evaluated. Mean age of the participants was 15 years (range 12-17 years) with a mean weight of 53.8 kg (range 45.1 – 79.8 kg). Overall, the FDC of DOR/3TC/TDF was well tolerated through 24 weeks. There was a low incidence of drug-related AEs (2.2%) with 95% CI (0.1-11.8), no drug-related SAEs or AEs ≥Grade 3 and no treatment discontinuation due to AEs. In the virologically-suppressed on stable ART participants there were no protocol-defined virologic failures and HIV-1 RNA <50 copies/ml was maintained at 95.3% with a 95% CI [84.2, 99.4]. One of the 2 ART-naïve participants achieved Escalating Real-Time Adherence (TERA) intervention compared to standard of care (SOC) on VS and electronic dose monitored adherence of antiretroviral therapy (ART), among viroemic (HIV-1 RNA=200 copies/ml) youth (ages 13-24 yrs) in the United States.

Methods: 89 YLWH were randomized to TERA intervention versus SOC and followed for 48 weeks with study visits at weeks 0, 4, 12, 24, 36 and 48. Remote coaching sessions were delivered at Weeks 0, 4 and 12, with continuous EDM monitoring for delayed or missed ART doses and as needed outreach from coach by text and phone in the TERA arm. Primary outcome was VS at week 12 (HIV-1 RNA <200 cp/ml at 10-14 weeks). RNA ≥ 200 cp/ml (10-14 wks) or missing set to failure. Proportions with VS were compared by arm (Fisher’s exact test and log binomial regression for adjusted comparisons). Secondary outcomes included EDM adherence summarized in 12-week intervals using percent days device was opened (PCT12) and incidence rates (IR) of number of ≥7-day gaps between openings (GAPIR), compared using Wilcoxon rank sum tests. Results are reported using data collected before the study paused due to COVID-19 in March 2020.

Results: 88 YLWH completed study entry; 55% male, 85% Black/African American, median age 22 years (range 13-24 yrs), 44% acquired HIV perinatally and 30% on ≥3rd ART regimen. VS was achieved in 15/43 (35%) (95% CI: 21%, 51%) TERA arm and 16/45 (36%) (95% CI: 22%, 51%) SOC arm participants; difference (TERA-SOC) was -1% (95% CI: -21%, 20%). No differences by arm were apparent at weeks 24, 36 or 48 on after adjusting for sex, age or mode of transmission. Of 54 participants with opportunity for follow-up to week 12, 14% (4/29) and 8% (2/25) in the TERA and SOC arms, respectively, achieved consistent VS (TERA – SOC: 6%; 95% CI: 15%, 25%). Median (Q1, Q3) PCT12 over the first 12 weeks was 72% (47%, 89%) versus 41% (21%, 59%) in the TERA and SOC arms, respectively (p<0.001). GAPIRs were higher in the SOC arm than TERA arm with SOC/TERA IR ratio of 2.51 (95% CI: 1.90, 3.33).

Conclusion: The 12-week TERA intervention improved adherence to ART but not VS among YLWH failing treatment. TERA will be further assessed for timing, timing, and outcome duration in YLWH.

603 RANDOMIZED CONTROLLED TRIAL OF AN ADHERENCE INTERVENTION IN YOUTH LIVING WITH HIV
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Background: Adolescent with HIV-1, between the ages of 12 and 18 years and weighing at least 45 kg who were antiretroviral therapy (ART)-naive or virologically-suppressed on stable ART, were enrolled into an open-label trial evaluating the once daily FDC tablet regimen of DOR 100mg, 3TC 300mg, and TDF 300mg. Safety, virologic and PK data were evaluated through Week 24 of therapy.

Results: 102 (52% male) adolescents were enrolled. Median age at entry was 15 years with a nadir CD4 count of 288 cells/mm³ (12.2%), with 58% receiving TDF/ FTC. At week 48, 94 of 102 subjects (92.2%) maintained virologic suppression, with no significant change in CD4 counts. Six subjects had virologic failure, 2 had RPV-associated mutations (K101E and Y181C). Significant decreases in total cholesterol, triglyceride, HDL and LDL (p<0.001) occurred at weeks 24 and 48. No significant changes in EFV-related symptoms, health-related QOL and depression score were observed; although there was significant improvement in performance-based assessments of executive function at week 24.

Conclusion: More than 90% of adolescents switching from EFV- to RPV maintained virologic suppression after 48 weeks. RPV was well tolerated and associated with improvements in metabolic profiles, executive and cognitive function.
virologic suppression by Week 24 and the other experienced protocol-defined virologic failure related to adherence issues. DOR geometric mean steady state trough concentration at Week 4 was 747 nM and subsequent concentrations were above the lower efficacy bound (>560 nM) for adults with HIV receiving 100mg QD DOR. PK for 3TC and TFV from the FDC were consistent with reported PK in adults receiving each drug individually.

Conclusion: At Week 24 the PK, safety, and tolerability of DOR as an FDC of DOR/3TC/TDF in adolescents were comparable to data reported in adults.

Overall virologic efficacy in the trial showed favorable antiretroviral effect comparable to data reported in adults.

605 ABACAVIR DOSING IN NEONATES FROM BIRTH: A PHARMACOKINETIC ANALYSIS

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Background: Antiretroviral treatment (ART) from birth in neonates (<28 days of age) at high risk of HIV acquisition can provide both enhanced HIV prophylaxis and early treatment. Although abacavir (ABC) is a recommended component of 1st line ART in children, pharmacokinetic (PK) data and dosing information are limited for neonates. ABC is licensed for children >3 months of age (8 mg/kg, BID), while the WHO recommends weight band dosing for children ≥4 weeks weighing ≥3 to <5.9 kg (60 mg or ~10 – 20 mg/kg, BID). We performed a PK analysis using ABC plasma concentrations from neonates and young infants to determine ABC dosing for term neonates.

Methods: Data were pooled from 3 studies administering ABC liquid: (1) PACTG 321 (2) a Tygerberg cohort and (3) IMPAACT P1106. Studies 1 and 2 performed intensive PK sampling in term neonates receiving ABC for HIV prophylaxis. Study 3 performed sparse PK sampling on term and low birth weight (LBW; <2500g) infants with HIV initiating ABC based ART after 1 month of life. ABC PK parameters were estimated using a population approach. Monte Carlo simulations were run for virtual term neonates to achieve ABC exposures (AUC0-24) within the expected range based on WHO weight band dosing (3.2 to 25.2 mcg.hr/ml).

Results: Forty-five infants contributed 308 ABC concentrations; 21 term neonates <15 days of life undergoing intensive PK sampling. LBW infants were older at first PK assessment with a median (range) postnatal age (PNA) of 78 (41–190) days and weight of 3.6 (2.4–5.8) kg. ABC plasma concentrations were described by a 1-compartment model. ABC CL/F was allometrically scaled according to infant body weight and PNA described maturation in a non-linear manner. At birth, term neonates demonstrated a low ABC CL/F of 0.15 L/hr/kg reaching 0.71 L/hr/kg by 6 months of age (~five-fold increase). ABC CL/F in LBW infants at 6 weeks PNA was similar to term infants of a similar chronological age. Simulations predicted that an ABC dose of 2 mg/kg BID in term neonates and then 4 mg/kg BID from 4–12 weeks of age, achieved an AUC0-24 in the expected range (Fig 1).

Conclusion: ABC elimination is greatly reduced at birth but rapidly increases over the first weeks of life. Our proposed mg/kg dosing for ABC from birth to 3 months of life provides exposures within the expected range, but data on LBW infants are needed. Using the WHO weight band dose of 60 mg for children ≥4 weeks and weighing ≥3 to <5.9 kg would lead to higher exposures, but no safety concerns have been reported.

606 DOSE OPTIMISATION OF LONG-ACTING INJECTABLES IN NEONATES VIA PBPK MODELLING

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Background: As we move towards the use of LA injectables for HIV treatment & prevention, there is great interest in the potential of these formulations for neonates & children. However, clinical trials in paediatric patients, especially neonates, are often impeded by logistical & ethical barriers. PBPK modelling can be applied to inform the selection of new therapeutics at appropriate doses. The objective of this study was to evaluate intramuscular (IM) & oral CAB in neonates & identify an appropriate initial dosing regimen to rapidly achieve therapeutic levels using mechanistic PBPK modelling.

Methods: A previously published whole-body neonatal PBPK model was modified to simulate CAB in neonates. The ontogeny of the key enzyme UGT1A1 was refined and validated using observed neonatal raltegravir clinical data. For further validation of model input parameters, IM & oral CAB were simulated in an adult PBPK model; observed, adult clinical data were used for comparison. Since depot release in the neonate is unknown, simulations were performed using the adult release rate (4.5x10-4) as well as with this parameter decreased by 2, 5 and 10-fold. The possibility of an oral safety lead-in in conjunction with an IM injection was also explored in the model.

Results: Several scenarios were modelled in healthy neonates with the aim of achieving plasma exposures 4-fold above the reported protein adjusted (PA) IC50 (4*PAIC50: 0.664 ug/mL). Early CAB concentrations & time to achieve target concentrations were sensitive to the IM release rate. The initial simulations of IM CAB suggested that a delay of 35 hours (Regimen 1, Table 1) is required to reach target concentrations if the infant CAB release rate is identical to adults. To overcome this lag a single-dose oral lead-in of CAB was simulated (Regimen 8, Table 1).

Conclusion: Though long-acting formulations have many advantages their utility in special populations such as neonates is still in question. This study evaluated the pharmacology of injectable IM CAB in neonates. Assuming the CAB depot release rate in neonates is the same as observed in adults, our simulations suggest a 20mg (4.4–6.7 mg/kg) IM injection alongside a single dose of oral CAB both initiated on day 0, is suitable to achieve target exposure (>4*PAIC50). However, since the effect of neonatal physiology on the depot release rate in neonates and establish an appropriate neonatal CAB dosing regimen.

Figure 1: Predicted abacavir exposure (AUC0-24) from birth to 6 weeks of age for a 3 kg term neonate when ABC is administered at the proposed dose (40,000 infants were simulated across the age range)
607 OPTIMIZING DOLUTEGRAVIR INITIATION IN NEONATES USING POPULATION PHARMACOKINETIC MODEL

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Background: Dolutegravir (DTG) is a commonly used ARV in pregnant women with HIV. Limited data are available on the pharmacokinetics of placenta-coupled DTG in infants born to mothers receiving DTG. For infants exposed to HIV, optimized DTG dosing during the first days of life may depend upon (i) the time of the last maternal dose prior to delivery and (ii) the time of DTG initiation after birth. The current study utilized population pharmacokinetic (popPK) modeling and simulation to optimize initiation of DTG in neonates.

Methods: IMPACT P10265 evaluated DTG PK during pregnancy (2nd, 3rd trimester) and post-partum in patients receiving 50mg daily. Paired cord blood and maternal concentrations at delivery along with neonatal washout concentrations were collected. Maternal data were combined in a popPK model. Monte Carlo simulations were utilized to generate maternal DTG concentrations at delivery (last maternal dose 6, 12, and 24 hr prior to delivery) in 3000 virtual mother-infant pairs. Paired cord blood to maternal plasma ratios were used to estimate neonatal DTG concentrations at birth and an additional sequential simulation performed to estimate neonatal pre-dose and CL/F concentrations following a 5 mg dose administered 0, 24, 48, or 72 hr after birth.

Results: Thirty-one maternal subjects and 18 neonates contributed data to the analysis. A total of 552 maternal concentrations were utilized to develop the maternal popPK model. The median apparent clearance (CL/F) in the third trimester was 1.06L/hr, 40% higher than post-partum. Seventy infant washout samples were utilized for the neonatal popPK model. The estimated half-life was 44.1 hr. The median ratio of cord blood to maternal plasma concentrations at delivery was 1.25 (1.07 – 1.40 [IQR]). The maternal and neonatal popPK models were utilized in sequential simulations and are summarized in Table 1.

Conclusion: Neonatal DTG concentrations at birth varied considerably based on the time of the last maternal dose prior to delivery and exhibited a slow decline over the first few days of life. Initiating infant DTG at 24-48 hrs after birth is appropriate when the last maternal dose was given within 24 hours of delivery. PopPK modeling and simulation can help evaluate neonatal DTG concentrations and dosing regimens to guide future clinical trials.

Table 1. Median Infant Concentration Pre and Post 5mg Dose

<table>
<thead>
<tr>
<th>Infant Time after Birth to 5 mg Dose (hours)</th>
<th>At Birth</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Time from Last Dose to Delivery (hours)</td>
<td>Infant Median Pre-dose Concentration (mg/mL)</td>
<td>Infant Median Max (mg/mL)</td>
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<td></td>
</tr>
<tr>
<td>6</td>
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<td>24</td>
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<td>0.78</td>
<td>0.50</td>
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*Adolescents: 18-21 years old. #Average daily dose was 4 mg over the course of 28 days, ALL averages were under 20 mg over 28 days simulations.
SAFETY AND PHARMACOKINETICS OF VRC01LS AND 10-1074 AMONG CHILDREN IN BOTSWANA

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Background: Broadly neutralizing monoclonal antibodies (bNAbs) suppress HIV-1 RNA and may deplete viral reservoir. Before evaluating dual bNAbs as a treatment alternative in children, we evaluated the safety and pharmacokinetics (PK) of intravenous VRC01LS and 10-1074 in early-treated children with HIV on suppressive antiretroviral treatment (ART).

Methods: Eligible children had received ART from <7 days through ≥96 weeks of life, and had HIV-1 RNA <40 copies/mL for ≥24 weeks prior to enrollment. The initial PK phase (A) (previously presented) evaluated safety and PK of each bNAb separately in two groups of 6 participants for 12 weeks. In the second phase (B), we evaluated safety and PK of the two bNAbs in combination, and with a higher VRC01LS maintenance dose based on review of Phase A. Six participants received IV infusions every 4 weeks of both 10-1074 (30 mg/kg) and VRC01LS (30 mg/kg) first dose followed by 15 mg/kg maintenance. PK samples were collected over 8 weeks and safety evaluated through 32 weeks. PK concentrations were measured by ELISA. Results were combined from both phases for analysis by noncompartmental (first dose Cmax, Cmin, and trough for 10-1074) and population PK (PopPK) methods. For the PopPK analysis, a two-compartment model was developed for each bNAb using NONMEM and 5000 virtual participants were simulated to predict steady-state concentrations.

Results: There were no infusion reactions, no expedited adverse events, and no grade 3 or 4 events related to dual bNAb administration through 32 weeks. The first dose median (range) Cmax and trough for VRC01LS were 776 (559-846) mcg/mL and 230 (158-294) mcg/mL, and first dose median (range) Cmax and trough for 10-1074 were 1405 (876-1999) mcg/mL and 133 (84-319) mcg/mL. All participants’ average concentrations following the first dose were >245 mcg/mL for VRC01LS and 290 mcg/mL for 10-1074. The population PK model was developed for each bNAb using NONMEM and 5000 virtual participants were simulated to predict steady-state concentrations.

Conclusion: IV infusions of VRC01LS and 10-1074 were well tolerated as dual therapy in children, and generated concentrations similar to those following single bNAb administration. Monthly dosing of VRC01LS at 15 mg/kg and 10-1074 at 30 mg/kg achieve target concentrations at steady state.

CANCER INCIDENCE IN HIV-POSITIVE CHILDREN: THE SAM STUDY, SOUTH AFRICA (2004-2014)

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Background: HIV is a known carcinogen and people living with HIV are at an increased risk for cancer compared to the general population. There is limited data available on the burden of cancer in HIV-positive children, particularly in sub-Saharan Africa. We aimed to determine cancer incidence in a national cohort of HIV-positive children (aged 0-14 years) in South Africa.

Methods: The South African HIV Cancer Match (SAM) study used privacy-preserving record linkages to create a national cohort of people living with HIV with cancer outcomes from national laboratory and South African National Cancer Registry data. We included children aged 0-14 years old at their first HIV-related laboratory record within the South African public sector health laboratories between 2004 and 2014 who had at least two distinct HIV-related laboratory records. We defined time under observation from the date of the first HIV-related laboratory test to cancer diagnosis or to last known HIV-related laboratory record. We calculated crude cancer incidence rates per 100,000 person-years.

Results: A total of 313,097 HIV-positive children were included in the study. In 802,030 person-years of follow-up, 743 incident cancers were diagnosed for an overall cancer incidence rate of 92.6/100,000 person-years. The majority (58.1%) of all diagnosed cancers were in males with an incidence rate of 118.0/100,000 person-years (95% confidence interval [CI]: 104.3-131.7) [Table 1]. Children with CD4 counts between 50-99 cells/μL at baseline and those aged 10-14 years had the highest cancer incidence rates at 137.9/100,000 person-years (95% CI: 95.2-199.7) and 121.4/100,000 person-years (95% CI: 104.6-140.9) respectively.

Conclusion: In countries with low HIV-prevalence, leukaeimias and lymphomas are the most common childhood cancers. In our study, Kaposi sarcoma is the most common cancer in HIV-infected children, as similarly seen in adults living with HIV. The higher cancer incidence rates in children aged 10-14 years were likely due to delayed HIV diagnosis with prolonged periods of immunosuppression and exposure to other oncogenic viruses. Additional analysis to determine risk factors for cancer incidence among this age-group is needed.

134 CANCER INCIDENCE IN HIV-POSITIVE CHILDREN: THE SAM STUDY, SOUTH AFRICA (2004-2014)

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1National Health Laboratory Service, Johannesburg, South Africa, 2University of Bern, Bern, Switzerland, 3Swiss Tropical and Public Health Institute, Basel, Switzerland

Background: HIV is a known carcinogen and people living with HIV are at an increased risk for cancer compared to the general population. There is limited data available on the burden of cancer in HIV-positive children, particularly in sub-Saharan Africa. We aimed to determine cancer incidence in a national cohort of HIV-positive children (aged 0-14 years) in South Africa.

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611 GROWTH DEFICITS ARE ASSOCIATED WITH AIRFLOW OBSTRUCTION IN PERINATILY ACQUIRED HIV

Engi F. Attia1, Wendy Yu2, Denise Jacobson1, Elizabeth Maleche-Obimbo3, Paige L. Williams4, Andrew Colin5, Meyer Kattan6, Sherry Eskander6, Michael H. Chung7, Kristina Crothers8, for the Pediatric HIV/AIDS Cohort Study (PHACS) 1University of Washington, Seattle, WA, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3University of Nairobi, Nairobi, Kenya, 4University of Miami, Miami, FL, USA, 5Columbia University, New York, NY, USA, 6Coptic Hope Center, Nairobi, Kenya, 7Emory University, Atlanta, GA, USA

Background: Chronic lung disease (CLD) is an emerging comorbidity among youth living with perinatally-acquired HIV (YPHIV) globally. Obstructive CLD, particularly with irreversible airflow obstruction (AFO) such as with obliterative bronchiolitis and poorly controlled asthma, may be an important subtype.

Methods: We performed a cross-sectional analysis of YPHIV (10-21 years old) in the Kenyan BREATHE (n=204) and US Pulmonary Complications in the Pediatric HIV/AIDS Cohort (PCPK; n=188) studies. Sociodemographic, clinical, immune function, and spirometry data were ascertainment within 3 months of enrollment. AFO was defined as a z-score < -1.64 for the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (GLI 2012); irreversible AFO also required post-bronchodilator FEV1 increase ≤10%. We fit modified log-binomial models using generalized estimating equations with robust variances to estimate prevalence ratios (PR) of AFO by country. Wasting and stunted growth were defined as BMI- and height-for-age z-score < -2, respectively.

Results: Kenyan YPHIV were younger, had more wasting and stunting, and had lower CD4/CD8 (Table) despite 99% current antiretroviral therapy use in Kenyan YPHIV. Of Kenyan YPHIV, 24 (12%) had AFO and 13 (7%) had irreversible AFO compared to 26 (14%) and 19 (6%) US YPHIV, respectively. Among Kenyan YPHIV, stunted growth was associated with AFO (PR=2.60 [95%CI 1.25-5.41], p=0.01); wasting was associated with irreversible AFO (4.62 [1.39-13.42], p=0.01). Among US YPHIV, stunted growth was associated with irreversible AFO (3.27 [1.12-9.56], p=0.03); lower CD4/CD8 was associated with AFO (2.17 [0.99-4.76], p=0.08) and irreversible AFO (2.86 [0.93-8.99], p=0.07). We detected no associations with tobacco smoke exposure.

Conclusion: The prevalence of AFO and irreversible AFO was similar in Kenyan and US YPHIV. Deficits in growth parameters were associated with AFO, including irreversible AFO, in both Kenyan and US YPHIV. Immune imbalance was consistent with a greater likelihood of AFO among US YPHIV. Our findings suggest that these CLD manifestations are similar across settings despite differences in risk factor prevalence, highlighting an urgent need to elucidate common pathways in CLD among YPHIV.

Table. Determine characteristics of Kenyan BREATHE and US PCPK study participants

<table>
<thead>
<tr>
<th>Measure</th>
<th>Kenyan YPHIV</th>
<th>US YPHIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at spirometry (years)</td>
<td>14.9 (12.7, 18.3)</td>
<td>14.7 (12.7, 18.4)</td>
</tr>
<tr>
<td>Female sex</td>
<td>92 (45%)</td>
<td>103 (48%)</td>
</tr>
<tr>
<td>CD4 T-cell count (cells/mL)</td>
<td>672 (289, 909)</td>
<td>334 (249, 733)</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.74 (0.38, 1.51)</td>
<td>0.91 (0.65, 1.25)</td>
</tr>
<tr>
<td>Active tobacco smoking</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Passive tobacco smoke exposure</td>
<td>47 (23%)</td>
<td>18%</td>
</tr>
<tr>
<td>Wasting (BMI-for-age z-score &lt; -2)</td>
<td>35 (19%)</td>
<td>8%</td>
</tr>
<tr>
<td>Stunted growth (height-for-age z-score &lt; -2)</td>
<td>44 (22%)</td>
<td>20%</td>
</tr>
<tr>
<td>FEV1 z-score, pre-bronchodilator</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>FEV1:forced expiratory volume in 1 second (pre-bronchodilator)</td>
<td>0.93 (0.58, 1.52)</td>
<td>0.70 (0.45, 1.08)</td>
</tr>
<tr>
<td>Data are presented as median (interquartile range) or %</td>
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613 ASSOCIATIONS OF GUT MARKERS WITH BODY FAT IN YOUTH WITH PERINATILY ACQUIRED HIV

Sahera Dirajal-Fargo9, Denise Jacobson1, Wendy Yu2, Ayesha Mirza3, Mitchell E. Geffner4, Jennifer Jao2, Grace A. McComsey5, for the Pediatric HIV/AIDS Cohort Study (PHACS) 1Case Western Reserve University, Cleveland, OH, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3University of Florida, Gainesville, FL, USA, 4Kubawe Clinical Research Center, Kinshasa, Uganda, 5Ann & Robert H Lurie Children’s Hospital of Chicago, Chicago, IL, USA, 6University Hospitals Cleveland Medical Center, Cleveland, OH, USA

Background: In adults with HIV, fibrotic and inflammatory pathways are associated with cardiac dysfunction. It is not clear whether these associations are related to aging and lifestyle. We hypothesized that evaluating proteins in plasma samples may be used to determine association between individual proteins with MPI status after FDR adjustment for multiple comparisons and multivariable models adjusted for age and body surface area were constructed for significant proteins. A lasso regression was used to also construct a model most discriminate of abnormal MPI.

Methods: The mean age of the study population was 14.3 years (IQR 8.1). In the univariable model, 18 proteins were significantly associated with MPI status after FDR adjustment. Of these, 5 remained significant in multivariable models: ST2 (adverse cardiac remodeling, OR 1.8 CI 1.2, 2.6), EN-RAGE (pro-inflammatory associated with coronary heart disease, OR 1.7 CI 1.2, 2.4), CHI1 (inflammatory mediator associated with heart failure with preserved ejection fraction OR 1.5 CI 1.0, 2.0), and FGF-21 (metabolic homeostasis and insulin sensitivity, OR 1.5 CI 1.0, 2.0) (Figure 1). In an orthogonal approach, the Lasso regression also identified these four primary proteins as well as COL1A1, PAGA, C5, TIMP4, IL-6RA, IL-17A, CCL16, CST5, and TNFRSF10C (AUC 0.818, CI 0.756, 0.880).

Conclusion: Using proteomics profiling in a unique cohort of children and young adults perinatally infected with HIV from Kenya, we identified proteins reporting on cardiac remodeling, inflammation and metabolism with higher circulating levels in young individuals with subclinical cardiac dysfunction. These results suggest that pathways dysregulated in overt adult HIV-related cardiovascular disease may also be dysregulated early in the HIV process and thus could serve as biomarkers for those at greatest risk of future cardiovascular disease.
with HIV RNA ≤1,000 copies/mL within 3 months of both DXA. Plasma levels of zonulin (a marker of intestinal permeability), intestinal fatty-acid binding protein (I-FABP, a marker of gut epithelial integrity), and lipopolysaccharide binding protein (LBp, a marker of microbial translocation) were measured by ELISA, within 6 mo of the first DXA. We assessed the association of baseline log_{10} transformed gut markers with total body fat and trunk fat at baseline and at 2 yr using linear regression models adjusted for potential confounders (Black vs. non-Black, Tanner stage, and sex).

**Results:** 261 youth were included; 128 had a second DXA. Median age at the time of first DXA (Q1, Q3) was 12 yr (10, 14), 49% were female, and 67% were Black. Median CD4 cell count was 761 cells/mm³; 90% had HIV RNA < 400 copies/mL; 49% were on PI-, 16% on NNRTI, 35% on other ART. Only 1 received InSTI-based ART. Baseline median (Q1, Q3) percent total body fat was 21.58% (14.94, 29.24) and trunk fat 41.28% (36.84, 45.96). The median percent increase in total body and trunk fat at 2 yr was 24.83% (8.70, 47.32) and 31.45% (12.16, 58.57), respectively. Distributions of I-FABP, LBp, and zonulin were not significantly different by CART or baseline CD4 cell count. In adjusted analyses, I-FABP was inversely related to percent total body fat at baseline and 2 yr, whereas LBp and zonulin were positively associated with total body fat at both time points (Table); no gut markers were associated with changes from baseline in total body or trunk fat.

**Conclusion:** Despite viral suppression, intestinal damage and the resultant bacterial translocation may influence body composition in YPHIV. Further studies are needed to investigate the role of gut dysfunction on body composition in YPHIV and elucidate underlying mechanisms.

<table>
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<th>Outcome</th>
<th>Unadjusted</th>
<th>Adjusted</th>
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**Methods:** We included 47,279 CHIV (<15 years old) on ART within 2004-2017 at 72 sites in Africa, Asia, the Caribbean, and Central/South America. We used mixed-effect Poisson regression models to estimate mortality by age, sex, CD4 at ART start, time on ART, region, and calendar period (using linear splines). In an adjusted analysis, we modified the data before model-fitting by simulating mortality outcomes for a further 6 months in those identified as LTFU, based on a Gompertz survival model fitted to leDEA tracing study data (n=221), and combined results of multiple simulations using Rubin’s rules.

**Results:** In the unadjusted analyses, 1,217 deaths were recorded during 183,989 years of observation. Amongst African CHIV <5 years old, mortality was highest in the first 6 months of ART, lower at 0.5-1 year (rate ratio: 0.37; 95% CI: 0.31-0.43), and lowest at >1 year (0.15; 0.06-0.36) of ART. Compared to CHIV 3-4 years old, mortality was twice (2.24; 1.93-2.60) and 6-fold higher (5.67; 4.71-6.82) in 2-3 and <1-year-olds, respectively. Compared to CHIV with a CD4 <5% at ART start, mortality halved with CD4 5-10% (0.57; 0.52-0.63), with further reductions at higher CD4%. Results were similar for regions outside of Africa. In CHIV 5-14 year olds, associations between mortality and ART duration or CD4 were similar. Fitted temporal trends indicate an average 68% reduction in mortality from 2005 to 2017 (33%-90% by region and risk group). Adjusted mortality estimates were approximately double unadjusted ones in 2017 (Figure), with the average reduction from 2005 now 40%.

**Conclusion:** Mortality among CHIV has decreased over time, after controlling for lower mortality at longer ART durations, higher CD4 values and older ages. However, mortality estimates in recent years doubled when accounting for worse outcomes among those LTFU, reducing these apparent temporal improvements.

614 GLOBAL TRENDS IN PEDIATRIC ON-TREATMENT MORTALITY ADJUSTED FOR LOSS TO CARE BIASES

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**Background:** UNAIDS projections of pediatric HIV prevalence and deaths rely on the International epidemiology Databases to Evaluate AIDS (leDEA) Consortium for mortality estimates among children with HIV (CHIV) receiving antiretroviral therapy (ART). Previous estimates may no longer be accurate due to expanded pediatric HIV care and treatment, and the possibility of unreported deaths in CHIV considered lost to follow-up (LTFU). We estimated all-cause mortality and its trends using both leDEA observational data from CHIV in routine care, and (ii) novel data from leDEA tracing studies that determined mortality outcomes in a sample of CHIV LTFU.

615 MORTALITY IN CHILDREN & YOUTH (<25 YEARS) WHO STARTED ART BUT ARE LOST TO CARE

**Patience Nyakato,1 Momra Comelli,1 Benedikt Christ,1 Nanina Andergoue,2 Josephine Mueharte,1 Laura Jefferys,1 Janneke Van Dijk,3 Monique Van Lottow,2 Cleophas Chimbedete,4 Sam J. Pithur,5 Michael J. Vinikoor,6 Matthias Egger,5 Marie Ballif,1 Mary-Ann Davies1

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**Background:** Despite significant progress in addressing HIV/AIDS, children, adolescents and young adults living with HIV (CAYHIV) continue to have poorer outcomes than adults aged ≥25 years. Loss to follow up (LTF) in antiretroviral therapy (ART) programs is a major challenge to achieving optimal treatment outcomes and the UNAIDS 2030 target. We aimed to compare site-reported mortality with mortality among patients confirmed LTF who were traced.

**Methods:** We included routine observational data on patients initiating ART between 2004-2017 who were not traced, and data obtained from tracing studies for those who were traced from five countries (Lesotho, Malawi,
616 MICROBIOLOGICAL FEATURES AND FOLLOW-UP OF NEOBATES BORN TO MOTHERS WITH COVID-19

Sara Vígil Vázquez1, Iztay Carrasco2, Alba Pérez Pérez2, Alicia Hernanz-Lobo2, Ángela Manzanarez2, Elena Márquez2, Olga Sanz2, Beatriz Pérez-Seoane3, Álvaro Solaz4, María Concepción Ortiz Barquero2, Monica Riaza2, Marta Pareja5, Manuel Sánchez-Luna6, María Luisa Navarro7, for GENSEO-COVID

1Hospital General Universitario Gregorio Marañón, Madrid, Spain, 2Hospital San Pedro de Alcántara, Córdoba, Spain, 3Hospital Reina Sofia, Tudela, Navarra, Spain, 4Hospital Universitario Infantia Sofia, San Sebastián de los Reyes, Madrid, Spain, 5Hospital Universitario La Fe, Valencia, Spain, 6Hospital Universitario de Badajoz, Badajoz, Spain, 7Hospital Universitario HM Monegros, Madrid, Spain, 8Complejo Hospitalario Universitario de Albacete, Albacete, Spain

Background: Literature evaluating the effect of SARS-CoV-2 infection in exposed newborns during pregnancy is still scarce. Although a 3% rate of perinatal transmission has been described, there is not enough evidence of viral transmission in biological samples through microbiological techniques. Our aim is to describe perinatal transmission in newborns exposed to SARS-CoV-2 during pregnancy and their follow-up.

Methods: The study period is from March 15 to November 30, 2020. Exposed newborns of SARS-CoV-2 infected mothers (with microbiologically confirmed COVID-19 disease during pregnancy or delivery) were included at 13 hospitals in Spain. Demographic, clinical and microbiological data were collected. Biological samples including nasopharyngeal swab, blood, urine, and meconium from newborns and blood, placenta, and breast milk from mothers were collected for reverse transcription polymerase chain reaction (RT-PCR) analysis.

Results: 282 exposed to SARS-CoV-2 neonates were recruited; 130 cases during the first wave (March 15-July 31) and 152 during the second one (August 1- November 30). The premature birth-rate was 20% and 13% respectively. Overall, eleven newborns were positive for RT-PCR in nasopharyngeal swab, eight of them during the first 24-48 hours after birth. Three of them presented viral load in urine sample and another three in meconium sample. Only one RT-PCR was positive in maternal blood samples (1/115) and placenta (1/81). All newborn blood samples collected at delivery were negative for RT-PCR (0/70). There was no viral load either in breast milk samples (0/79). Placental immunohistochemistry performed for SARS-CoV-2 showed no virus (0/16). Two newborn death were described none of them related to SARS-CoV-2. Those newborns exposed to SARS-CoV-2 were asymptomatic and with normal weight and psychomotor development at 6-months follow-up.

Conclusion: Intrauterine SARS-CoV-2 transmission seems unlikely, describing a 3.9% rate of neonatal infection after delivery. A high rate of prematurity is described, mostly during the first wave. SARS-CoV-2 can be detected by RT-PCR in urine and meconium of neonates with positive nasopharyngeal RT-PCR, whereas it has not been detected in any newborn blood. The detection in maternal blood and placenta was anecdotal and it was not detected in breast milk samples. Except for the complications derived from prematurity, exposed newborns evolution was satisfactory.
both with D614G. Flow cytometry was applied to measure S-specific memory B cells using fluorochrome-conjugated recombinant S, and S-specific IL-2, IL-17, TNF-α, or IFN-γ producing T cells after stimulation with overlapping peptides of full-length S.

**Results:** No adverse effects were observed with either vaccine. Plasma S-specific IgG responses were induced by both vaccines at wk 4, increased after the second dose, and persisted through wk 14 (Fig 1A). All S regions were targeted by plasma IgG (Fig 1B), and RBD-specific IgG was also detected in saliva. Serum antibodies achieved >95% ACE2 blocking by wk 6 (1/10 dilution), remaining >90% at wk 14. Geometric mean ID50 titers of neutralizing antibodies in the PSA exceeded 10[3] from wk 6 through wk 14 (Fig 1C) and strongly correlated with whole virion neutralization (p<0.0001). In the protein vaccine group, S-specific CD27+ memory B cells peaked at 3.1% (mean) of total memory B cells; and S-specific CD4+ T cell responses were dominated by IL-17 and IFN-γ. Mean S-specific CD27+ B cells peaked at 0.9% total memory B cells in mRNA vaccines and S-specific CD4+ T cells produced IL-2, IFN-γ, IL-17, or TNF-α. The S-2P-3M-052-SE and mRNA-LNP vaccines were well-tolerated and highly immunogenic in infant Rhesus Macaques, with persistent IgG binding and neutralization responses that are comparable to those reported for adult RMs and humans. Our results provide proof-of-concept that a pediatric SARS-CoV-2 vaccine could induce long term protection against SARS-CoV-2.

**Conclusion:** The S-2P-3M-052-SE and mRNA-LNP vaccines were well-tolerated and highly immunogenic in infant Rhesus Macaques, with persistent IgG binding and neutralization responses that are comparable to those reported for adult RMs and humans. Our results provide proof-of-concept that a pediatric SARS-CoV-2 vaccine could induce long term protection against SARS-CoV-2.

**Figure 1:** S-specific plasma IgG responses in infant Rhesus Macaques after S-2P protein (blue triangles) or S-2P mRNA-LNP (red circles) vaccination. (A) AUC (log10) copies/10^6 cells. (B) Spike-specific responses expressed as log10, MFI (C) ID50 (log50) values of neutralizing antibodies at wks 0, 4, 6, 8, 10 and 14. Symbols and lines represent individual animals. Arrows indicate vaccinations.

### 619 DIAGNOSTIC ACCURACY OF SARS-CoV-2 ANTIGEN RAPID TEST COMPARED TO REAL TIME PCR IN PED

**Serena Villaverde**, Sara Domínguez-Rodríguez, Gema Sabrido, MP Romero, Marta Plata, Ana Belén Jiménez, Marta Arana, Begoña Pérez-Moneo, Cinta Moraleda

**Methods:** The agreement between the two methods was calculated using Cohen’s kappa index.

**Results:** A total of 1620 patients were tested in 7 hospitals. The overall sensitivity for RAT and RT-PCR was 35.4% (95% CI, 1.8–2.1) and 1.8% (95% CI, 1.6–1.9), and specificity was 99.8% (95% CI, 99.4–99.9). The positive predictive value (PPV) for this 4.8% prevalence was 92.5% (95% CI, 78.6–97.4). The negative predictive value (NPV) was 97.3% (95% CI, 96.8–97.8). The positive likelihood ratio (PLR) was high - 233.8 (IC 95%, 73.3–743.3), and negative likelihood ratio (NLR) was low - 0.54 (95% CI, 0.44–0.67)

**Conclusion:** Compared to RT-PCR, the sensitivity of the RAT and RT-PCR was low in children with <5 days of symptoms of COVID-19. The specificity and PLR were good, and the NLR and concordance with RT-PCR were only moderate.

These results suggest that the test is very good when the result is positive, and that the test has only a limited value when the result is negative. In relation with screening and public health policy, these results should be interpreted considering also rapidness, availability and false positives ratio compared to RT-PCR or other tests.

### 620 CLINICAL SYNDROMES CAUSED BY COVID-19 AND A BAYESIAN MODEL TO PREDICT SEVERITY

**Alfredo Tagarro**, Sara Domínguez-Rodríguez, Serena Villaverde, Miquel Serna-Pascual, Francisco José Santantonio, Jesus Saavedra-Lazano, Victoria Fumadó, Cristina Epalza, Jose Antonio Alonso, Paulo Rodríguez-Molina, Joan Miquel Pujol, Cinta Moraleda

**Methods:** We conducted a multicenter, prospective study of children aged 0 to 18 years old with SARS-CoV-2 infection in 52 Spanish hospitals. The primary outcome was the need for critical care: defined as the combined outcome of admission into a PICU, and/or need for respiratory support beyond nasal prongs. To understand the probability of needing critical care according to the diagnostic group and for each risk factor, a Bayesian multivariable model was applied. To build a predictive model of critical care, a naive Bayes algorithm was implemented in a web app.

**Results:** 292 children were hospitalized from March 12th, 2020 to July 1st, 2020; of them, 214 (73.3%) were considered to have relevant COVID-19 (r-COVID-19). Among patients with r-COVID-19, 24.2% needed critical care. Out of 214 patients, 22.4% were admitted into a pediatric intensive care unit, and/or need for respiratory support beyond nasal prongs. To understand the probability of needing critical care according to the diagnostic group and for each risk factor, a Bayesian multivariable model was applied. To build a predictive model of critical care, a naive Bayes algorithm was implemented in a web app.

**Background:** The accuracy of rapid antigen tests (RAT) SARS-CoV-2 for children is unknown. Our aim was to determine the diagnostic accuracy and concordance of the RAT and RT-PCR in nasopharyngeal smear (NPS) samples, in symptomatic pediatric population.

**Methods:** This is a descriptive, retrospective, multicentre clinical study nested in a prospective, observational, multicenter cohort study. We included pediatric patients aged 0 to 16 years with symptoms consistent with COVID-19 of ≤5 days of evolution, attended in the Emergency Departments of the seven centers involved. A total of two consecutive NPS were obtained from each patient: one was employed to perform the RAT and the other to perform RT-PCR. Sample size for a non-inferiority study was calculated considering 80% power, for a 5% prevalence and a 90% sensitivity, using RT-PCR as the gold standard reference. A confusion matrix was displayed. Non-inferiority of sensitivity and specificity between diagnostic tests was assessed using the Mc Nemar’s test.

**Results:** A total of 214 patients were tested in 7 hospitals. The overall sensitivity for RAT and RT-PCR was 45.4% (95%CI, 34.1–57.2), and specificity was 99.8% (95% CI, 99.4–99.9) (Figure 1). The positive predictive value (PPV) for this 4.8% prevalence was 92.5% (95% CI, 78.6–97.4). The negative predictive value (NPV) was 97.3% (95% CI, 96.8–97.8). Positive likelihood ratio (PLR) was high - 233.8 (IC 95%, 73.3–743.3), and negative likelihood ratio (NLR) was low - 0.54 (95% CI, 0.44–0.67).

**Conclusion:** Compared to RT-PCR, the sensitivity of the RAT and RT-PCR was low in children with <5 days of symptoms of COVID-19. The specificity and PLR were good, and the NLR and concordance with RT-PCR were only moderate.

These results suggest that the test is very good when the result is positive, and that the test has only a limited value when the result is negative. In relation with screening and public health policy, these results should be interpreted considering also rapidness, availability and false positives ratio compared to RT-PCR or other tests.

**Figure 1:** Specificity between diagnostic tests was assessed using the McNemar’s test.
Conclusion: We described the spectrum of r-COVID-19 in hospitalized children, consisting of 4 large syndromes of decreasing severity: MIS-C, bronchopulmonary syndrome, gastrointestinal syndrome, and a mild syndrome with complications. The risk factors increase the risk differently depending on the syndrome. A Bayesian model was implemented in an online app to anticipate the individual risk of critical care.

Results: Among COVID-19, majority had SARS-CoV-2-specific CD4+ (100% spike, 83% non-spike) and CD8+ (85% spike-containing, 83% non-spike) T-cells. There was a trend for lower frequencies of AIM+ T-cells to all peptide MP in MIS-C, with significantly lower responses to non-spike antigens in CD4+ (p<0.05) and CD8+ (p<0.05) T-cells compared to those in COVID-19. In addition, COVID-19 had higher reactivity to stimulation, with significantly greater SI for spike CD4+ T-cell responses compared with HC (4.62 vs 1.93, p<0.05) and non-spike compared to both MIS-C (3.27 vs 1.44, p<0.05) and HC (3.27 vs 1.60, p<0.01). Interestingly, most HC also had detectable CD4+ (70% spike, 50% non-spike) and CD8+ T-cells (90% spike, 75% non-spike) against SARS-CoV-2 antigens, possibly attributable to prior infection by endemic coronaviruses. RBD IgG levels were similar between MIS-C and convalescent COVID-19.

Conclusion: We find more robust CD4+ and CD8+ T-cell responses against non-spike SARS-CoV-2 peptides in convalescent COVID-19 compared to MIS-C. Equivalent humoral responses against spike RBD among MIS-C and COVID-19 suggest that impaired SARS-CoV-2-specific T-cell response to non-spike antigens may contribute to the immunopathogenesis of MIS-C.

621 ELUCIDATING SARS-CoV-2 T-CELL RESPONSES IN PEDIATRIC COVID-19 AND MIS-C

Vidisha Singh1, Veronica Obregon-Perko1, Stacey A. Lapp1, Anna M. Homer1, Alyssa Brooks1, Lisa S. Mac Coy1, Laila S. Hussaini1, Austin Lu1, Theda Gibson1, Evan Anderson1, Christina A. Rostad1, Ann Chahroudi1
1Emory University, Atlanta, GA, USA

Background: Multisystem Inflammatory Syndrome in Children (MIS-C) can develop 1–2 mo post SARS-CoV-2 infection. MIS-C is characterized by fever, multiorgan dysfunction requiring hospitalization, and systemic inflammation. To evaluate a potential role for aberrant T-cell responses as a potential mechanism for MIS-C pathogenesis, we quantified SARS-CoV-2-reactive T cells in children with COVID-19, MIS-C, and healthy children (HC).

Methods: Hospitalized children ages 0–20 yrs with COVID-19 (n=13) or MIS-C (n=18) were enrolled from May–Sep 2020. Peripheral blood mononuclei ar cells (PBMC) were obtained from convalescent phase of infection (28–54 d from illness onset) for COVID-19 or at hospitalization for MIS-C to approximate similar time since infection. Plasma SARS-CoV-2 receptor binding domain (RBD) antibody titers were determined by ELISA. PBMC from HC (n=20) with undetectable RBD antibodies served as controls. T-cell responses were quantified using activation-induced marker (AIM) assay after stimulation with SARS-CoV-2 peptide “megasapols” (MP); CD4 MP_S with 253 spike-spanning peptides, CD4 MP_R with 221 remaining non-spike; spike-containing CD8 MP_A and non-spike CD8 MP_B with 314 each. Frequency of AIM+ T-cells and stimulation index (SI) were compared across donor groups.

Results: Among COVID-19, majority had SARS-CoV-2-specific CD4+ (100% spike, 83% non-spike) and CD8+ (85% spike-containing, 83% non-spike) T-cells. There was a trend for lower frequencies of AIM+ T-cells to all peptide MP in MIS-C, with significantly lower responses to non-spike antigens in CD4+ (p<0.05) and CD8+ (p<0.05) T-cells compared to those in COVID-19. In addition, COVID-19 had higher reactivity to stimulation, with significantly greater SI for spike CD4+ T-cell responses compared with HC (4.62 vs 1.93, p<0.05) and non-spike compared to both MIS-C (3.27 vs 1.44, p<0.05) and HC (3.27 vs 1.60, p<0.01). Interestingly, most HC also had detectable CD4+ (70% spike, 50% non-spike) and CD8+ T-cells (90% spike, 75% non-spike) against SARS-CoV-2 antigens, possibly attributable to prior infection by endemic coronaviruses. RBD IgG levels were similar between MIS-C and convalescent COVID-19.

Conclusion: We find more robust CD4+ and CD8+ T-cell responses against non-spike SARS-CoV-2 peptides in convalescent COVID-19 compared to MIS-C. Equivalent humoral responses against spike RBD among MIS-C and COVID-19 suggest that impaired SARS-CoV-2-specific T-cell response to non-spike antigens may contribute to the immunopathogenesis of MIS-C.

622 ASYMPTOMATIC SARS-CoV-2 CHILDREN HAVE LOWER INFECTIVITY AND INTACT MEMORY RESPONSES

Nicola Cotugno1, Alessandra Ruggiero1, Giuseppe R. Pascucci1, Bonfante Francesco2, Maria Raffaella Petraro2, Bernardi Stefania2, Donato Amadio2, Piccioni Livia3, Daniele Donà3, Maria Raffaella Petrara3, Carla Giaquinto3, Carlo Concato3, Petter Brodin4, Paolo Rossi5, Anita De Rossi5, Paolo Palma5
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Background: SARS-CoV-2 (CoV-2) infected children often range from being paucysymptomatic to fully asymptomatic. The impact of this population on the epidemics due to their ability to transmit the virus and achieve protective immunity has been poorly defined. We explored CoV-2 infectivity potential and anti-CoV-2 cellular (CD8, NK and B) and humoral response in symptomatic (SY) and asymptomatic (ASY) CoV-2 infected children, screened for a family member resulted infected.

Methods: CoV-2 viral load was measured by RT-PCR and digital droplet PCR (ddPCR) on longitudinal samples of nasopharyngeal swabs in 9 AS and 33 SY (samples were paired according to symptoms’ onset for SY and first family contact for AS). Virus infectivity was tested by Virus focus forming assay (FFA). CoV-2 antibodies were investigated by Diasorin (CoV-2 Ab) and Ab-mediated neutralization activity (PRNT) at diagnosis, (samples collected >5 days from symptoms onset in SY, or from first family contact in AS were excluded from this timepoint), and in the convalescent phase (CP) (10–14 days after infection). Cellular response was analyzed by flow cytometry: 1) Ag-specific B cells, by a S1+2 CoV2-R-PE probe; 2) Ag-specific CD8+ T cells by ICAM+; 3) natural-killer (NK) phenotype. Mann-Whitney was used for comparison; linear regression was used to evaluate the associations between virus load and infectivity.

Results: ASY showed lower viral load (p=0.004) and faster virus clearance (p=0.0002) compared to SY. Virus infectivity was associated with ddPCR (rho=0.66; p=0.002). ASY and SY showed similar levels of CoV-2 Ab and PRNT, at both diagnosis and at follow up. During the CP, the proportion of CoV-2 Ab negative was 33.3% for both groups and PRNT was negative in 16.6% and 15.7% of AS and SY respectively. Anti-CoV-2 cellular immunity was comparable between ASY and SY. Indeed Ag-specific B cells and CD8 T cells were detectable despite immunosupiomy and no major differences were found between the groups. Total NK frequency was similar between the groups, while a regulatory NK subset (CD56bright NK cells) was higher in AS compared to SY (p=0.01).

Conclusion: These data show that AS have a lower infectivity potential compared to SY suggesting that mitigated restrictive measures or alternative screening may be considered for this population. In addition, these patients showed an intact ability to produce humoral and cellular CoV-2 specific responses hence contributing to achieve herd immunity as much as SY.
HIGH SPECIFIC IMMUNE RESPONSE AND LOW T-CELL ACTIVATION IN CHILDREN WITH COVID-19

Maria Raffaella Petara, Bonfante Francesco, Paola Costenaro, Francesco Carmona, Anna Cantarutti, Elena Ruffoni, Daniele Donà, Costanza Di Chiara, Marisa Zanchetta, Luisa Barzon, Carlo Giaquinto, Anita De Rossi

1University of Padova, Padova, Italy; 2Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; 3Venezia Institute of Oncology IDIV, Padova, Italy; 4University of Milano–Bicocca, Milan, Italy

Background: SARS-CoV-2 infected children are often asymptomatic or paucisymptomatic compared to adults. The immune response plays a pivotal role in dictating the clinical outcome in infected adults, but it is still poorly investigated in the pediatric population.

Methods: Fifty-seven family clusters of SARS-CoV-2, attending the Department for Women’s and Children’s Health (University of Padova), were enrolled between March and September 2020, for a total 209 subjects. SARS-CoV-2 infection was confirmed in 155 patients (SARS+: 93 ≥15 years [group A]; 34 children ≥6-15 years [group B]; 28 children <6 years [group C]) by virus molecular detection and/or serology. In 41 available samples, measurement of SARS-CoV-2 levels (VL) was performed by an in-house quantitative One-Step ddPCR method. A blood sample was obtained at a median [IQR] of 2.8 [2.1-3.7] months after baseline (symptom’s onset and/or first positive virus detection). Neutralizing antibodies (Nabs) were detected by a Plaque Reduction Neutralization Test (PRNT). Activated CD8+ (CD38+HLA-DR+) and regulatory T cells (T-regs; CD4+Foxp3+CD127-CD25+) were analyzed by flow cytometry.

Results: VL did not differ by age (18507 [326-339315], 6723 [3427-114587], and 21106 [162-152500] copies/5µl, in group A, B and C, respectively; overall, p=0.955). Group C had the highest PRNT titer compared to the other groups (p<0.001; Figure c), Conversely, T-regs were inversely correlated with PRNT titer (group A: rs=-0.527 , p<0.0001; B: rs=-0.494 p=0.003; C: rs=-0.547 p<0.0001; Figure b). Activated CD8+ and regulatory T cells were significantly higher in group A compared to the other groups (p<0.001; Figure a), and were inversely correlated with PRNT titer (group A: rs=-0.527, p<0.0001; B: rs=-0.494 p=0.003; C: rs=-0.547 p<0.0001; Figure b). Conversely, T-regs were significantly higher in group C compared to the others (p<0.001; Figure c), and were positively correlated with PRNT values in children (group C: rs=0.662 p=0.0001; B: rs =0.532 p=0.001; A: rs=0.160, p=0.125; Figure d).

Conclusion: Levels of SARS-CoV-2 did not differ among age classes, but adults displayed a higher T cell activation and a lower production of anti-SARS Nabs than children. Conversely, younger infected children had the highest production of anti-SARS Nabs and the lowest non-specific T cell activation, most likely due to their higher expression of regulatory T cells.

HIGH AND PERSISTENT NEUTRALIZING ANTIBODIES IN CHILDREN RECOVERED FROM COVID-19

Bonfante Francesco, Paola Costenaro, Anna Cantarutti, Costanza Di Chiara, Chiara Cosma, Alessio Bortolami, Maria Raffaella Petara, Matteo Pagliari, Sandra Cozzani, Giovanni Di Salvo, Liviana Da Dahl, Luisa Barzon, Anita De Rossi, Daniele Donà, Carlo Giaquinto

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Background: Recent evidences suggest that SARS-CoV-2 neutralizing antibodies (Nabs) may persist over time, however lack of knowledge still regards the pediatric population.

Methods: A single-centre, prospective observational study evaluated family clusters of COVID-19 attending the Pediatric Department, University Hospital of Padua (Italy). Confirmed COVID-19 was defined by positive SARS-CoV-2 molecular detection and/or serology; patients/family symptom’s and virological positivity were considered to define the infection onset (baseline). Blood samples were analyzed in pair to detect Nabs through Plaque Reduction Neutralization Test (PRNT), and IgG through chemiluminescent immune-enzymatic assay (CLIA) MAGLUMI® 2000 Plus; IgG >1.1 kAU/L and/or PRNT≥1:10 were considered positive. SARS-CoV-2 viral load (VL) was quantified by multiplex quantitative assay based on One-Step RT-ddPCR. Geometric mean titers (GMT) and 95% Confidence Intervals of IgG/PRNT were evaluated, stratified by age and time from baseline to sample collection. Trends over time of immune-virological response were assessed. P-value <0.05 was considered statistically significant.

Results: Among 213 subjects (57 families) evaluated, 155 had confirmed COVID-19 including 73 (47%) children/older siblings of 8 years median age (IQR 4-13) and 82 (53%) parents aged 42 years (IQR 34-46); 93.5% had asymptomatic/mild COVID-19. From the cumulative analysis of 194 blood samples, Nabs persisted over a median period of 95 days (IQR 67-133) from baseline. Children showed significantly higher Nabs than older subjects, with children <3 years ranging from a 4-fold difference at 1-2 months to 8.8-fold difference at 3-6 months after baseline, compared to adults (table). The longitudinal assessment of 42 subjects sampled at 60 days (SD+/−9.9) and at 150 days (SD+/−24.2) showed a 2-fold increase in Nabs in children <6 years (PRNT 144, 95% C.I. 74.42-277.94 versus 303, 95% C.I. 196.43-468.57) and a substantial stability in Nabs among older subjects. CLIA IgG significantly decreased over time for all age classes, becoming negative in 13/42 subjects (31%), compared to 1/42 subjects detected by PRNT. Among 32 individuals checked for VL within 4 days from baseline, VL directly correlated with PRNT titers in subjects >15 years (Pearson Coefficient =0.70, p=0.0349) but not in pediatric cases.

Conclusion: Asymptomatic/mild COVID-19 disease triggers in children a superior and persistent humoral response compared to adults.
625 ROBUST HUMORAL IMMUNE RESPONSES TO SARS-CoV-2 INFECTION IN CHILDREN

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Background: The low susceptibility of children to severe illness with SARS-CoV-2 infection could be related to distinct virus-host interactions. Some studies indicate that SARS-CoV-2 infected adults with mild symptoms rapidly lose their antibody responses, but the kinetics of the antibody response in children have been less studied. To evaluate the antibody response of SARS-CoV-2 antibodies in infected children, we used samples from the Biobiospecimens from Respiratory Virus-Exposed Kids (BRAVE Kids) Study, a community-based prospective cohort study of children and adolescents with SARS-CoV-2 infection or exposure.

Methods: Samples from 71 SARS-CoV-2 infected children (median age: 9.7 years, IQR 4–16) collected at enrollment (M0), 2 and 4 months after exposure (M2, M4) were analyzed. A Luminex-based multiplex binding assay was used to measure Ig isotype (IgG, IgM, IgA) and IgG subclass (IgG1, IgG3) against 7 SARS-CoV-2 epitopes: whole spike (S), subunit 1 (S1), S2, receptor binding domain (RBD), N-terminal domain (NTD), nucleocapsid (NC) and membrane (M).

The ability of antibodies to block viral interaction with the human receptor ACE2 was evaluated by an ELISA-based assay, and neutralization was assessed in a pseudovirus assay.

Results: At time of enrollment (median of 5 days after infection), all participants had detectable levels of IgM and IgG against at least one of the tested SARS-CoV-2 antigens, and 91% had detectable IgG levels. IgM and IgG levels declined with time, although all children still had detectable levels of anti-S IgG and IgG at M4. In contrast, IgG binding to all viral regions increased significantly at M2 and, at M4, most children maintained robust IgG response.

Conclusion: SARS-CoV-2 infected children develop robust antibody responses that are still detectable 4 months after infection. This suggests that children could respond well to SARS-CoV-2 vaccination and highlights the need to test candidate vaccines in pediatric populations.

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Conclusion: SARS-CoV-2 infected children develop robust antibody responses that are still detectable 4 months after infection. This suggests that children could respond well to SARS-CoV-2 vaccination and highlights the need to test candidate vaccines in pediatric populations.
SARS-CoV-2 SeroPrevalence and IgG Levels Are Lower Among People Living With HIV
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1University of California San Francisco, San Francisco, CA, USA

Background: Although data are mixed, most cohorts show a similar or lower COVID-19 incidence among people living with HIV (PLWH) compared to the general population. However, incidence may be impacted by lower testing rates among vulnerable populations. We compared SARS-CoV-2 seroprevalence and IgG levels, and disease severity, among patients with and without HIV receiving care within a county hospital system over a three-month period.

Methods: From August through October 2020, remnant serum samples were collected from all PLWH who underwent routine outpatient laboratory testing at San Francisco General Hospital which houses a large HIV clinic (Ward 86). Patients with HIV were matched on time of collection (same day) and age (±5 years) to 1-2 adults without HIV. SARS-CoV-2 levels of IgG levels was quantified in serum using the Pylon IgG assay (100% specificity on internal validation).

Seroprevalence was compared by HIV status via conditional logistic models, adjusting for sex. For those with reactive results, IgG levels were compared by HIV status using log-transformed generalized estimating equations. Severe disease, assessed via chart review, was defined as requiring oxygen.

Results: Among 1,411 individuals (46% PLWH), the median age was 58 (IQR: 49–65), 64% were men. COVID-19 seroprevalence was 3.1% among PLWH, compared to 6.8% among people without HIV (adjusted odds ratio 0.41; 95% confidence interval (CI): 0.25–0.68, p<0.001). Among those with reactive COVID-19 IgG results (n=72, 20 in PLWH); antibody levels were 47% lower among PLWH (95% CI: 19–65%) lower; p=0.003; Figure); however, there was a trend towards higher disease severity among PLWH (15% (n=3) vs. 4% (n=2); p=0.13).

Conclusion: Both seroprevalence, and absolute SARS-CoV-2 IgG levels in those with reactive results, were lower among PLWH, within a time and age-matched population of outpatients receiving routine laboratory testing in an urban hospital. PLWH may have had higher adherence to non-pharmaceutical interventions (NPIs) than those without HIV, leading to lower COVID-19 seroprevalence and, possibly, lower COVID-19 IgG levels if infected with a lower viral inoculum due to NPIs. Alternatively, PLWH may mount lower antibody responses to SARS-CoV-2, as has been demonstrated with hepatitis B and yellow fever vaccines. Further studies of COVID-19 susceptibility and immunity are needed among PLWH. Moreover, PLWH should be enrolled in SARS-CoV-2 vaccine studies or followed after vaccination to ensure they mount sufficient humoral responses.
Table 1: New cases of COVID-19 among patients in DTP (people living with HIV) and patients on PrEP from March to November 2020

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629 SEROPREVALENCE OF ANTIBODIES AGAINST SARS-CoV-2 AMONG PEOPLE LIVING WITH HIV (PLWH)

Smitha Gudipati1, Monica Lee1, Megan Scott1, Sean Yaphe1, Nicholas Yared1, Indira Brar1, Joanne Huisting1, Norman Markowitz1

Background: COVID-19 first reported in the US on 1/2020 is a global pandemic. In PLWH, COVID-19 outcomes have been reported to be similar or worse compared to the general population; however, the seroprevalence in this group has not been identified. As of 6/2020, 2.7% of the 960 PLWH in our Ryan-White (RW) clinic have tested PCR+ for COVID-19. Yet, these likely represent only a fraction of COVID-19 infections, as an unknown proportion of cases are mild or asymptomatic and not diagnosed. Our goal was to estimate the seroprevalence in our RW patients (pts), irrespective of known past COVID-19 infection. The RW program funds HIV care for a diverse group impacted by a number of social determinants of health, including low socioeconomic status.

Methods: We conducted a seroprevalence study, which recruited pts in the RW program at Henry Ford Hospital. All RW pts were offered participation during clinic visits. After informed consent, pts completed a survey and had blood sampled for COVID-19 antibody using the Beckman Coulter Access SARS-CoV-2 IgG assay. Pts’ electronic medical records were reviewed for demographic clinical features, including previous COVID-19 testing. The study was IRB approved.

Results: 187 PLWH were enrolled from 9/2020-11/2020 (Table 1). Median age: 46 (IQR: 34-57); 153 males; 152 black; 24 reported a previous COVID-19 exposure; 66 had a BMI of >/= 30. Mean CD4 count was 629.5 (IQR: 390-859), and 129 pts were HIV suppressed. 17 had PCR-confirmed COVID-19, and 16 reported past infection by PCR+ in the same population. This estimate of past infection is also limited as some cases were catch up tested 6-8 months from the initial PCR test.

Conclusion: The COVID-19 seroprevalence of 13% reported in this study of PLWH in our clinic was about 5-fold greater than the number of reported cases by PCR+ in the same population. This estimate of past infection is also underestimated given the absence of antibody at the time of the serological testing in 53% of PLWH with documented PCR+ infection and the likelihood of infection in some of those never tested. Conversely, the impact of health disparities on the RW pts likely increases the chance of acquisition of COVID-19 compared to other populations. In order to better understand the penetration of COVID-19 into the PLWH community, a greater understanding of the dynamics of the antibody response to COVID-19 is needed.

Table 3: Results of Exposure Questions Performed in 187 People Living with HIV in the Ryan-White Clinic

<table>
<thead>
<tr>
<th>Mean Age (yr, IQR)</th>
<th>Patients COVID-19 IgG negative (N=447)</th>
<th>Patients COVID-19 IgG positive (N=45)</th>
</tr>
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<tbody>
<tr>
<td>Sex (M:F)</td>
<td>229:14</td>
<td>3:1</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td>Black: 123</td>
<td>White: 14</td>
</tr>
<tr>
<td></td>
<td>Hispanic: 14</td>
<td>Other: 10</td>
</tr>
<tr>
<td>Occupation (%)</td>
<td>Healthcare worker: 7</td>
<td>First responder: 2</td>
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<td></td>
<td>Restaurant industry: 12</td>
<td>Grocery store: 4</td>
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<tr>
<td></td>
<td>Other/unspecified: 135</td>
<td></td>
</tr>
<tr>
<td>Rural Metabolic Index (BMI)</td>
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<td>3.5 (1.1-6.9)</td>
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<tr>
<td></td>
<td>18.5-20.9</td>
<td>16.0-20.9</td>
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<tr>
<td></td>
<td>25-30</td>
<td>16.0-20.9</td>
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<tr>
<td></td>
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<td>18.5-20.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CD4 (CD4+/CD3+)</td>
<td>253 (200-315)</td>
<td>253 (200-315)</td>
</tr>
<tr>
<td></td>
<td>Median HIV-1 viral load (pg/mL, IQR)</td>
<td>Median HIV-1 viral load (pg/mL, IQR)</td>
</tr>
<tr>
<td>Known Exposure to COVID-19 (%)</td>
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<td>23 (5)</td>
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<tr>
<td>COVID-19 EPRP (%)</td>
<td>Positive PCR, Symptomatic</td>
<td>Negative PCR, Symptomatic</td>
</tr>
<tr>
<td></td>
<td>9 (2)</td>
<td>16 (3)</td>
</tr>
<tr>
<td></td>
<td>PCR+ not performed, Symptomatic</td>
<td>15 (3)</td>
</tr>
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</table>

Conclusion: HCWs exposure risks continue to persist in the workplace and in the home. We observed few positive molecular tests, suggesting few transmission, but these exposure may potentially sustain seropositivity. These findings are preliminary and need to be further investigated.

Figure 1: Losses regression curve of change in antibody levels among seropositive (A) and seronegative (B) healthcare providers in NYC.
631 OVERCROWDED HOUSING INCREASES RISK FOR COVID-19 MORTALITY: AN ECOLOGICAL STUDY

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1Vancouver Coastal Health, Vancouver, Canada, 2Thomas Jefferson University, Philadelphia, PA, USA

Background: The 2019–novel coronavirus (COVID-19) has devastated the United States (US) population and has exacerbated existing health inequalities. Those residing in areas of high population density are at an elevated risk, suggesting that residence in an overcrowded household may result in heightened vulnerability. However, as the association between residing in an overcrowded household and risk of mortality from COVID-19 is unknown, the purpose of this study was to analyze this relationship.

Methods: COVID-19 data was acquired for each of the 85 cities in Los Angeles County, the region with the highest number of recorded cases in the US, along with data on housing and demographics. Overcrowded households were defined as having 1.0+ persons per room. Bivariate regression was performed between the number of overcrowded households and the number of COVID-19 deaths. Backwards stepwise linear regression was then conducted with risk factors for COVID-19 mortality as potentially eligible input variables. Collinearity was assessed by considering the variance inflation factors (VIF); variables with high collinearity (VIF above 8) were removed.

Results: Bivariate regression indicated that the number of overcrowded households was positively associated with the number of COVID-19 deaths (standardized β = 0.844, p < 0.001). COVID-19 case totals, number of individuals aged 60 or above, and number of overcrowded households met conditions for inclusion in the backwards stepwise linear regression model. This analysis revealed that all three of these independent variables were positively associated with number of deaths, with the largest effect being seen with overcrowded housing (standardized β = 0.386, p = 0.001), followed by number of cases (standardized β = 0.307, p = 0.014), and number of individuals aged 60+ (standardized β = 0.282, p < 0.001).

Conclusion: Overcrowded housing was found to be a major risk factor for COVID-19 mortality in this study and served as a better predictor of number of deaths than the number of people 60+, and even the total number of COVID-19 cases. These findings have important implications for addressing the COVID-19 pandemic. While age and comorbidities have frequently been described as risk factors for poor outcomes, these findings indicate a critical need for COVID-19 control efforts to more thoroughly assess for overcrowded housing. Furthermore, the striking absence of research on overcrowded housing indicates a clear direction for future studies.

632 DYNAMICS OF THE COVID-19 EPIDEMIC AT THE CALIFORNIA-MEXICO BORDER

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Background: As countries around the world review interventions for containing the COVID-19 pandemic, movement of populations has been identified as a key factor of viral dispersal and limiting the population flow intensity has been assessed by considering the variance inflation factors (VIF); variables with high collinearity (VIF above 8) were removed.

Methods: All publicly available SARS-CoV-2 full genome sequences (human host) available on the GISAID database were collected (as of Nov. 16th, 2020). After sequence curation, a multistep phylogenetic approach was applied to identify putative clusters of transmission within CA (across counties), MX (across states) and across the MX/CA border. These clades were analyzed with a discrete phylogeographic model to evaluate transmission dynamics of COVID-19 in the MX/CA region.

Results: From a total of 174,324 SARS-CoV-2 sequences including 5,471 sequences from Mexico (7 States, n=223)/California (29 counties, n=5,248), we identified 622 unique introduction events into the study region, including 381 clusters of size ≥3 from ≥2 locations (i.e. CA county and/or MX state). Of these, 339 (89%) clusters were from CA only across 23 counties, 5 (1.3%) were from MX only across 6 states and 38 (10%) included sequences from both CA and MX. Discrete phylogeographic analysis revealed a complex viral migration network within CA/MX and across the border (Figure 1A, left panel). Analyses of the 38 clusters including sequences from CA and MEX showed bidirectional migration events across the border (Figure 1B). In particular we showed migration events in the border region from the border state of Baja California, MX to the border county of San Diego, CA and from the border county of Imperial County, CA to the border state of Sonora, MX (Figure 1A, right panel).

Conclusion: This comprehensive analysis of all publicly available COVID-19 sequences showed local transmission across regions within CA and MX as well as across neighboring locations across the border. Similar to the 2009 H1N1 pandemic, the MX/CA border does not appear to be a major barrier to the spread of COVID-19, necessitating coordinated transnational intervention approaches.

Figure 1. A. Relative contribution of California counties and Mexico States to the spread of COVID-19 in the border region. Results accounting for migration links associated with an adjusted Bayes factor (BFa) ≥ 3. The Sankey plot represents the proportion of migration events from each source risk group (‘from’) to the recipient risk group (‘to’). B. Number of introductions into California from Mexico and into T Mexico from California.

633 A DESCRIPTION AND ANALYSIS OF COVID-19 IN A POPULATION-REPRESENTATIVE COHORT

Yannis Herrmann1, Tim Starck1, Niall Brindl2, Philip J. Kitchen1, Lukas Raedeker1, Jakob Sebastian1, Lisa Koeppe1, Frank Tobian1, Aurelia Souares1, Andre Mihaljevic1, Utte Merle1, Theresa Hippchen1, Felix Herth1, Andreas Welker1, Claudia Denninger1

Background: Most data on COVID-19 was collected in hospitalized cases. Much less is known about the spectrum of disease in entire populations including non-hospitalized patients and minors. In this study, we examine a representative cohort in an administrative district in Southern Germany who tested positive for SARS-CoV-2 between February and June of 2020.

Methods: We contacted all confirmed SARS-CoV-2 cases in an administrative district in southern Germany. Consenting participants answered a retrospective survey either via a telephone, electronically or via mail. Clinical and sociodemographic features were compared between hospitalized and non-hospitalized patients. Additionally, we assessed potential risk factors for hospitalization and time to hospitalization in a series of regression models. As predictors we assessed age as a continuous variable, sex, smoking as a continuous variable using pack years, living with children (age <18), hypertension (yes/no), coronary heart disease (CHD; yes/no), diabetes (type 1 or 2; yes/no) and lung conditions (yes/no). Lung conditions were defined as a combined variable of either COPD, asthma treated with medications, any other lung disease or previously performed lung surgery. Secondly, we estimated...
the influence of the same covariates on the time from symptom onset to hospitalization with a Cox proportional hazard ratio (HR) model. 

**Results:** We included 897 participants in our study, 65% out of 1,305 total cases in the district with a mean age of 47 years (range 2-97), 51% of which were female and 47% had a pre-existing illness. The percentage of asymptomatic, mild (symptomatic, no hospitalization), moderate (leading to hospital admission) and critical illness (requiring mechanical ventilation) was 24 patients (6%), 713 (79%), 97 (11%) and 16 (2%), respectively. Seventeen patients (2%) died. The most prevalent symptoms were fatigue (65%), cough (62%) and dysgeusia (60%). The risk factors for hospitalization included older age (OR 1.05 per year increase; 95% CI 1.04-1.07) preexisting lung conditions (OR 3.09; 95% CI 1.62-5.88). Female sex was a protective factor (OR 0.51; 95% CI 0.33-0.77).

**Conclusion:** This population-representative analysis of COVID-19 cases confirms age, male sex and preexisting lung conditions but not cardiovascular disease as risk factors for severe illness. Almost 80% of infection take a mild course, whereas 13% of patients suffer moderate to severe illness.

---

**Table 1: Characteristics of Symptomatic and Asymptomatic Participants by SARS-CoV-2 Status**

<table>
<thead>
<tr>
<th>Status</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>25-34</td>
<td>&lt;15 years old</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Preexisting Illn</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Death</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

---

**Figure:**

- **SARS-CoV-2 Surveillance in 14 Health Facilities in Malawi**
- **SARS-CoV-2 Transmission Among First Cases and Their Contacts in Kisumu County, Kenya**
Conclusion: Within 3 months from the first identified case of SARS-CoV-2 infection in Kisumu County, office workers had the highest risk of infection, suggesting a need for more rigorously applied physical distancing and masking policies as employees of non-essential services return to work. While transmission from cases to contacts was relatively low, the vast majority occurred within households and children were disproportionately represented among secondary cases. Enhanced support for within-household distancing during the isolation of cases may be needed. Given the concurrent increase in office-worker infections, undetected community transmission outside our enrolled cohort likely occurred.

636 CUMULATIVE INCIDENCE OF SARS-CoV-2 INFECTION AND EPIDEMIC METRICS, UNITED STATES
Patrick S. Sullivan1, Kayoko Shioda1, Eric Hall1, Heather Bradley1, Nicole Luisi1, Kristen Nelson1, Travis Sanchez1, Mariah Valentine-Graves1, Mansour Fahimi1, Rich Rothenberg1, Ben Lopman1, Aaron J. Singer1, for the COVID Vu Study Group
1Emory University, Atlanta, GA, USA, 2Georgia State University, Atlanta, GA, USA, 3Marketing Systems Group, New York, NY, USA

Background: Understanding the cumulative incidence of SARS-CoV-2 infections in the United States has been limited by asymptomatic infections, waning antibodies after natural infection, incomplete case ascertainment and reporting, and limited representative samples. We conducted a probability survey of US households to measure SARS-CoV-2 infection and immune response and to estimate the cumulative incidence of SARS-CoV-2 infection.

Methods: A multistage random sample of US postal addresses were mailed a kit to self-collect an anterior nares swab and a dried blood spot (DBS) sample from August to December 2020. Specimens were tested by EUA-approved PCR and serology tests. Weighted estimates of antibody prevalence, together with historical patterns of antibody waning, were used to estimate the cumulative incidence of SARS-CoV-2 infections, the diagnosed fraction, and infection fatality rate (IFR). Weighted estimates were used to calculate prevalence ratios comparing demographic, geographic, and clinical subgroups.

Results: 37,056 kits were mailed to sampled US households. Overall, 5,666 surveys were completed by December 8, 2020; of these, 4,654 also returned a DBS specimen with a valid antibody result. Overall participation rate was 11.8%. We estimated 39,421,841 (95% credible interval (CrI): 33,759,801–43,958,068) total infections by October 30, 2020, an estimated diagnosed fraction of 17% (95% CrI: 15-23%) and an estimated IFR of 0.64% (95% CrI: 0.58-0.75%). Daily seroreconversion peaked by Sept 2020 and remained stable through November 2020 due to a balance of waning antibodies and new infections (Figure). Non-Hispanic Black (PR: 2.2; 95% confidence interval (CI): 1.2-4.0) and Hispanic (PR: 3.1, CI: 1.8-5.3) respondents were more likely than White non-Hispanic to have laboratory evidence of prior SARS-CoV-2 infection. Prevalence was also higher among those living in metropolitan areas (PR vs non-metropolitan areas: 2.5, CI: 1.3-5.0) and among those reporting cold or flu symptoms (PR: 2.6, CI: 1.6-4.1) or loss of taste or smell (PR: 12.8, CI: 8.5-19.4) since January 1, 2020.

Conclusion: We report the results of the first national probability sample of US households to assess the prevalence of antibodies to SARS-CoV-2 and cumulative incidence. As of October 30, 2020, about 1 in 8 US residents aged ≥18 years had been infected with SARS-CoV-2, and about 1 in 6 of those had been diagnosed. Household-based probability surveys provide a minimally biased benchmark to characterize epidemic dynamics.
US POPULATION-BASED SURVEY OF VACCINE WILLINGNESS AND SARS-CoV-2 ANTIBODY PREVALENCE

Aaron J. Siegler1, Eric Hall2, Travis Sanchez3, Heather Bradley1, Nicole Lu7i1, Kristen Nelson1, Mariah Valentine-Graves1, Mansour Fahimi2, Kayoko Shioda1, Ben Lopman1, Rich Rothenberg1, Patrick S. Sullivan1, for the COVIDu Study Group

Emory University, Atlanta, GA, USA, 1Georgia State University, Atlanta, GA, USA, 2Marketing Systems Group, Horsham, PA, USA

Background: Developing representative estimates of COVID-19 vaccine acceptance will be essential to public health planning as the vaccine supply moves towards sufficiency in meeting initial levels of demand. We conducted a national probability household survey to assess vaccine willingness and history of SARS-CoV-2 infection based on antibody response.

Methods: Study materials were sent to an address-based sample frame that includes nearly all residential addresses in the US. Participants completed a behavioral survey and dried blood spot (DBS) specimen collection for SARS-CoV-2 antibody testing during the study period, August 9 – December 8, 2020. Vaccine willingness was measured with a 5-point Likert scale item with responses ranging from “Very unlikely” to “Very likely.” Sample weights were calculated and applied to descriptive statistics and prevalence ratios (PR).

We categorized persons as either Ig negative, Ig positive and aware of prior COVID-19 infection, or Ig positive and unaware of prior COVID-19 infection.

Results: A total of 4,654 respondents completed the survey and had a valid antibody test result, representing 242,875,582 US adults. Overall, a substantial proportion, 32% (76,967,749 adults), were unsure or unwilling to receive a COVID-19 vaccine. Many groups at increased risk for SARS-CoV-2 had higher proportions unsure or unwilling, including Black (46%) relative to White (30%, p<0.01), persons working outside home (38%) relative to at home (21%, p<0.01), and smokers (44%) relative to nonsmokers (29%, p<0.01) (Table 1). Dissonance between transmission risk and vaccine willingness was also observed in biologic data. Persons Ig positive (previously infected) and unaware of their status had a higher point estimate of unwillingness to be vaccinated (39%) than persons Ig negative with no history of infection (31%, p=0.08). Overall, we estimate 12% (29,241,030 adults) were very unlikely to be vaccinated (39%) than persons Ig negative with no history of infection (31%, p<0.01).

Conclusion: In the first national probability survey with biomarker data, we demonstrated that many groups with higher risk for COVID-19 infection had lower willingness to take a COVID-19 vaccine. This finding is in accordance with pre-existing fault lines of inequity in our society. Substantial vaccine uptake promotion is needed, and should be targeted to address inequities correlated with vaccine willingness.

Table 1. Likelihood of taking a COVID-19 vaccine in national probability survey

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Survey n</th>
<th>Weighted n</th>
<th>Weighted %</th>
<th>95% CI</th>
<th>95% CI</th>
<th>Prevalence Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1,484</td>
<td>136,419,067</td>
<td>31.8</td>
<td>30.9-32.8</td>
<td>0.97</td>
<td>0.79-1.22</td>
<td>1.19</td>
</tr>
<tr>
<td>By Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>202</td>
<td>17,022,153</td>
<td>29.8</td>
<td>24.6-34.9</td>
<td>0.98</td>
<td>0.79-1.22</td>
<td>1.20</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>1,282,947</td>
<td>23.6-34.9</td>
<td>0.98</td>
<td>0.79-1.22</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>361</td>
<td>64,453,823</td>
<td>30.3</td>
<td>28.5-31.9</td>
<td>0.99</td>
<td>0.71-1.27</td>
<td>1.30</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>86</td>
<td>28.6</td>
<td>0.95</td>
<td>0.71-1.27</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>85</td>
<td>6,064,585</td>
<td>28.6</td>
<td>22.7-35.9</td>
<td>1.79</td>
<td>1.20-2.42</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Employment: Working outside the home: 683 (40,022,647 | 39.4 44-45 | 1.79 | 1.20-2.42 | 1.30 |

Smoking: No: 1,140 | 55,030,480 | 28.6 | 26.5-31.6 | 1.79 | 1.20-2.42 | 1.30 |

Serology: Ig Negative: 1,136 | 72,329,333 | 31.1 | 29.3-34.3 | 1.79 | 1.20-2.42 | 1.30 |

Table 2. Prevalence of SARS-CoV-2 antibodies among different groups

<table>
<thead>
<tr>
<th>Clade</th>
<th>G</th>
<th>GH</th>
<th>GR</th>
<th>S</th>
<th>V</th>
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<tbody>
<tr>
<td>Phase 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Phase 2</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Phase 3</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

PREVALENCE OF ANTI-SARS-CoV-2 ANTIBODIES IN SOUTH AFRICAN BLOOD DONORS

Wendy Sykes1, Russell Cable1, Charlie Coleman1, Tanya Glatt1, Eduard Grebe2, Laurette Mhlanga3, Nadia Pietersen1, Ronel Swanevelder3, Karin van den Berg4, Marion Vermeulen5, Alex Welte1

1South African National Blood Service, Johannesburg, South Africa; 2Western Cape Blood Service, Cape Town, South Africa; 3Vitallant Research Institute, San Francisco, CA, USA; 4South African Centre for Epidemiological Modelling and Analysis, Stellenbosch, South Africa; 5Western Cape Provincial Department of Health, Cape Town, South Africa

Background: Given the inevitable, likely substantial, under-ascertainment of SARS-CoV-2 infection at the population level, using routine laboratory testing, the prevalence of SARS-CoV-2 antibodies (seroprevalence) is an important marker of Covid-19 epidemiology. As nationally representative household surveys are a major undertaking, it will be important to find efficient ways to reliably estimate antibody prevalence from much simpler, less expensive protocols.

Methods: Subject to meeting standard blood donor eligibility criteria, and a standing opt-out arrangement for research use of specimens primarily obtained for blood safety screening, unsolicited blood donations, obtained on particular ‘collection days’ at 219 donation sites in South Africa, during January 2021, were tested for SARS-CoV-2 antibodies using the Roche Cobas e411 platform. Donors are currently requested to defer donation if they were diagnosed with Covid-19, or plan a trip to a疫区。
or experienced Covid-19-like symptoms, in the preceding 14 days. Estimates were stratified by age, sex and race. The study will have additional testing days. Phone interviews with both antibody positive and negative donors are being conducted to probe PCR diagnosis and symptoms.

**Results:** Tested donations numbered 4547, from donors aged 16-81. Seroprevalence did not vary significantly between sexes or age groups. Headline results for the main race groups (in South African nomenclature) are: Black 50.3% (95% CI 55.8 – 60.7%), White 13.8% (95% CI 12.3 – 15.4%), Asian 23.4% (95% CI 19.4 – 27.7%) and Coloured 36.2% (95% CI 31.4 – 41.1%). The population group weighted overall national estimate is then 51.4% (95% CI 49.4 – 53.4%). This is almost 25 times as high as the official prevalence, on 10 January, of having been diagnosed with Covid-19, namely 2.1%. See Figure.

**Conclusion:** These are the first relatively widely representative SARS-CoV-2 antibody prevalence estimates from South Africa. Population level representativeness of this methodology warrants further exploration – but it is worth noting that 1) at elsewhere, the obtained antibody prevalence estimates imply SARS-CoV-2 attack rates that are easily an order of magnitude higher than the apparently relatively uninformative official case counts; and 2) the marginal cost of performing this study, over the cost of routine blood bank operations, was almost entirely comprised of the cost of reagents. South African case fatality rate estimates will need significant revision.

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**A PROSPECTIVE CASE-COHORT STUDY OF COVID-19 IN PERSONS WITH HIV:**

**CoV19-19 STUDY**

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1Hospital Clinic of Barcelona, Barcelona, Spain, 2Hospital Ramón y Cajal, Madrid, Spain, 3Hospital San Pedro, La Roca, Spain, 4Hospital Costa del Sol, Marbella, Spain, 5Hospital Universitario Infanta Leonor, Madrid, Spain, 6Hospital del Mar, Barcelona, Spain, 7Hospital Universitario Príncipe de Asturias, Madrid, Spain, 8Hospital Universitario de La Princesa, Madrid, Spain, 9Hospital de Figueres, Figueras, Spain, 10Hospital Universitario Parc Taulí, Sabadell, Spain, 11Hospital Universitario de Bellvitge, Barcelona, Spain

**Background:** Several large cohort studies have shown that adults with HIV (PWH) may have worse COVID-19 outcomes than non-HIV-infected persons. Whether it may be due to a higher frequency of co-morbidities or to a direct HIV effect is currently unclear.

**Methods:** We performed a nation-wide multicenter prospective case-cohort study. Consecutive COVID-19 confirmed PWH (cases) admitted in 39 Spanish centers were matched 1:1 to COVID-19 confirmed non-HIV-infected adults (controls) for center, calendar week, age and gender. The contribution for death centers were matched 1:1 to COVID-19-confirmed non-HIV-infected adults (controls) for center, calendar week, age and gender. The contribution for death and death outcomes through 8 weeks post-admission. Hospital discharge and mortality were analyzed as competing risks using a multivariable cause-specific hazards model.

**Results:** Patients were 50.9% Black, 39.4% White and 9.7% other race; 11% were Hispanic. Mortality decreased markedly over time, with cumulative incidence (95% CI) 30 days post-admission of 19.1% (17.2, 21.3) in March-April versus 6.3% (4.3, 8.9) in July-September; 19% of deaths occurred after discharge. During this time, average age (SD) at admission declined from 62.7 (17.6) to 53.4 (20.6), ICU level care at admission increased from 16.5% to 18.6%, mechanical ventilation declined from 9.4% to 2.9%. Compared to Caucasian, Black race was associated with more severe disease at admission, a higher rate of co-morbidities and residence in low income zip code. In multivariable models, there were no detectable differences in mortality risk by race; while admitting hospital, increasing age, admission early in the pandemic, and severe disease and low blood pressure at admission were associated with increased mortality hazard (Figure 1). Mortality appeared similar between sexes, though males tended to have longer hospital stays, hospital length of admission declined from 9.8% to 2.9%

**Conclusion:** We found that morbidity and mortality for hospitalized COVID-19 patients substantially decreased over time but post-discharge mortality remained non-trivial. Black race was associated with more risk factors for morbidity and with treatment at hospitals with lower mortality. In multivariable models, there were no detectable race differences in hospital outcomes. Future work is needed to better understand the identified between-hospital differences in mortality.
Figure 1: Mortality hazard ratios (HR) from the multivariate cause-specific Cox regression (model 1, n = 2493).2

Table 1: Factors associated with time to viral suppression.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Level</th>
<th>HR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;40 yrs</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>≥40 yrs</td>
<td>1.17 (0.94 – 1.45)</td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td>Educational Level</td>
<td>≤ HS</td>
<td>Reference</td>
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</tr>
<tr>
<td>&gt; HS</td>
<td>0.95 (0.72 – 1.28)</td>
<td>0.725</td>
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</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>Reference</td>
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</tr>
<tr>
<td>Black</td>
<td>0.98 (0.91 – 1.13)</td>
<td>0.290</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Other race</td>
<td>Reference</td>
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<tr>
<td>Ethnicity</td>
<td>Hispanic/Latino</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>Other ethnicity</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>Site 1</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Site 2</td>
<td>2.27 (1.64 – 3.17)</td>
<td>0.0001***</td>
<td></td>
</tr>
<tr>
<td>Site 3</td>
<td>0.48 (0.23 – 1.00)</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Site 6</td>
<td>1.09 (0.30 – 3.80)</td>
<td>0.902</td>
<td></td>
</tr>
<tr>
<td>Site 7</td>
<td>1.10 (0.39 – 3.33)</td>
<td>0.859</td>
<td></td>
</tr>
<tr>
<td>Median Household Income</td>
<td>&lt; 35K</td>
<td>Reference</td>
<td></td>
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<tr>
<td>35K – 79K</td>
<td>0.75 (0.50 – 1.11)</td>
<td>0.190</td>
<td></td>
</tr>
<tr>
<td>79K – 100K</td>
<td>0.56 (0.32 – 0.99)</td>
<td>0.039</td>
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<tr>
<td>BMI Category</td>
<td>Normal</td>
<td>Reference</td>
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</tr>
<tr>
<td>Overweight</td>
<td>0.94 (0.44 – 2.01)</td>
<td>0.898</td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>1.60 (0.80 – 3.20)</td>
<td>0.126</td>
<td></td>
</tr>
<tr>
<td>Unweighted</td>
<td>3.04 (1.30 – 6.95)</td>
<td>0.003**</td>
<td></td>
</tr>
<tr>
<td>Month of Admission</td>
<td>Mar-Aug 2010</td>
<td>Reference</td>
<td></td>
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<tr>
<td>May-June 2009</td>
<td>0.70 (0.44 – 1.11)</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>Jul-Dec 2008</td>
<td>0.65 (0.39 – 1.05)</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>Normal</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.90 (0.55 – 1.45)</td>
<td>0.621</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1.03 (0.61 – 1.76)</td>
<td>0.916</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: Younger age, injection drug use, and stage 3 HIV were associated with worse VL patterns; people with these characteristics may need focused efforts to successfully enter HIV care. The findings can be used towards DC’s efforts to help HIV Epidemic by shortening the time from HIV diagnosis to ART initiation and VS. The absence of association with other demographic factors strongly suggests that factors not historically measured in surveillance should be examined to determine correlates of successful engagement in HIV treatment.
THE EPIDEMIOLOGY OF ADVANCED HIV DISEASE BEFORE AND AFTER UNIVERAL ART IN BOTSWANA

David S. Lawrence†, Mark W. Tenforde‡, Thandi Milton§, William Hurt∥, Hannah Mitchell¶, Kwanza Lechile∥∥, Fredah Mulenga∥∥∥, Charles Muthoga¶¶, Christopher G. Williams∥∥∥∥, Leah Owen∥∥∥∥, Mooketsi Moleb⁵⁶, Thebo P. Leeme⁵⁷, Julia Ngidi⁵⁸, Madisa Mine⁵⁹, Joseph N. Jarvis⁵⁹

1London School of Hygiene & Tropical Medicine, London, UK. 2University of Washington, Seattle, WA, USA. 3Botswana–University of Pennsylvania Partnership, Gaborone, Botswana. 4Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana. 5Botswana National Health Laboratory, Gaborone, Botswana. 6University of Botswana, Gaborone, Botswana

Background: The proportion of people living with HIV who have very advanced disease (CD4<100 cells/μL) has remained relatively constant over the past decade in sub-Saharan Africa, despite widened access to antiretroviral therapy (ART). We aimed to describe the characteristics of individuals presenting with very advanced HIV disease in Botswana before and after the introduction of universal ART and characterise the relationship between ART status and mortality.

Methods: We compared demographics and ART status in two cohorts of patients with CD4 counts <100 cells/μL recruited into a reflex cryptococcal antigen (CrAg) screening program before (2015/16) and after (2018/19) the introduction of universal ART in Botswana. Data were collected from sequential individuals undergoing CD4 count assessment at the Botswana Harvard HIV Reference Laboratory in Gaborone. Associations between 6-month mortality and ART status were determined using a Cox regression analysis adjusted for age, sex, and CD4 count.

Results: 1645 individuals were included in the 2015/16 cohort and 743 in the 2018/19 cohort. Median age and sex were similar between cohorts (37 vs 39 years, 50% male vs 55% male); median CD4 counts were 54 cells/μL (IQR 25-78) and 59 cells/μL (IQR 31-83). The 2018/19 cohort was significantly more likely of universal ART and characterise the relationship between ART status and mortality.

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Conclusion: The majority of patients in Botswana with very advanced HIV disease are now ART experienced and have previously been in HIV care. Mortality risk is highest among ART naïve individuals and those defaulting treatment, and declines rapidly once individuals are established on ART. These findings highlight the urgent need to identify individuals who have difficulty effectively engaging with HIV treatment services and improve their retention in care and adherence through differentiated service delivery models.

Impact of Aging on ART Continuation for Performance Management and Target Setting

Jessica Stephens†, Lana Lee‡, Noah Bartlett§, Melaku Dessie∥, Mary Mahy∥∥, George K. Siberry∥∥∥


Background: Retention of clients on HIV treatment is essential for achieving the second and third of the UNAIDS 90-90-90 goals. The President’s Emergency Plan for AIDS Relief (PEPFAR) requests programs to monitor age-specific retention using aggregated data, in order to identify demographic groups who may be experiencing treatment interruptions at higher rates. However, retention measures may be distorted by normal aging into and out of age bands. This analysis quantifies the impact of aging on PEPFAR retention estimates for eight PEPFAR priority countries: Kenya, Mozambique, Nigeria, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe.

Methods: We used data submitted to PEPFAR in 2018 and 2019 on people living with HIV (PLHIV) currently receiving antiretroviral therapy (ART) (TX_CURR), PLHIV newly enrolled on ART (TX_NEW), and UNAIDS Spectrum Modeling for the number of PLHIV on ART by one-year age band. We calculated proxy retention as currently calculated by PEPFAR [TX_CURR 2019 / (TX_CURR 2018 + TX_NEW 2019)] and compared it to the proxy retention but adjusted for aging (“aging adjusted PEPFAR retention” [AAPR]) [TX_CURR 2019 / (aging-adjusted TX_CURR 2018 + TX_NEW 2019)]. To calculate the aging-adjusted TX_CURR 2018, we predicted baseline ART in 2019 using Spectrum estimates for 2018. Next, we aggregated predicted baseline 2019 and 2018 in 2018 age band groups(<1, 1-9, 10-14, 15-19, 20-24, 25-29, 30-35, 35-39, 40-49, 50+). Finally, we took the proportion of predicted baseline 2019 relative to 2018, which we applied to the TX CURR 2018.

Results: After adjusting for aging, estimates of retention on ART increases for most age bands compared to the current PEPFAR retention proxy approach, except for 10-14 and >40. Adjusted for aging, proxy retention on average increased for <1 by 37.2% (AAPR: 73.8%); 1-9 by 3.3% (AAPR: 83.0%); 15-19 by 6.8% (AAPR: 81.5%); 20-24 by 6.0% (AAPR: 72.8%); 25-29 by 4.6% (AAPR: 76.1%); 30-34 by 3.1% (AAPR: 83.6%); and 35-39 by 1.2% (AAPR: 91.8%). Proxy retention adjusted for aging on average decreased for 10-14 by 2.8% (AAPR: 91.7%); 40-49 by 4.5% (AAPR: 98.7%) and 50+ by 10.0% (AAPR: 94.8%).

Conclusion: Normal aging can distort age-band retention estimates, especially at the extremes of ages. Assessment of retention for <1 age band needs special treatment, as all infants will age out over the course of one year. Treatment programs need systematic methods to account for aging to better assess and optimize continuity of care across the lifespan.

PATIENT TRANSFERS BETWEEN PRIMARY CARE ART SERVICES IN THE WESTERN CAPE, SOUTH AFRICA

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Background: People living with HIV (PLH) on antiretroviral therapy (ART) require longitudinal care, frequently delivered across multiple health facilities. However there are few data on how transfers of PLH between primary health care (PHC) facilities could affect ART adherence and retention.
Methods: We constructed a cohort of all patients on ART in the Western Cape province (2008–2018) using viral load (VL) data from the National Health Laboratory Service. VL testing is routinely available and recommended at 6 months after ART start and then yearly. Unique patient identifiers and probabilistic matching linked individuals across facilities; PLH >16y with >2 VLs in the study period (>1 VL at a PHC facility) were included; new patients were censored after 2016 to allow >2y follow-up. Transfers were defined as PLH with VLs recorded at >1 PHC facility. Transfer-out (TFO) was recorded as the last VL date at the original facility. Loss to follow-up at analysis closure (LTFU, no VL in the last two years of follow-up), disengagement with return to care (RTC, >2y between consecutive VLs), and VL>50 cps/mL were compared in those who did and did not TFO. We also described subsequent viremia (VL>50 cps/mL) among those with VL<50 cps/mL at TFO.

Results: Overall, 256,905 PLH were included in the analysis (69% female, median age 34y [IQR 28–40], median follow-up 4y [IQR 2–6]); 64,847 (25%) had >1 TFO between PHCs (Figure). Time from ART initiation to TFO was 13m (IQR 6–34) and 27% had a VL>50 cps/mL at TFO. Increased TFO rates were observed with: age <30y (adjusted rate ratio [aRR]; 95%CI: 1.31; 1.29–1.33), female sex (1.25; 1.23–1.27), first VL in a rural district (1.26; 1.24–1.29) and first VL>50 cps/mL (1.06; 1.05–1.08). Compared to PLH who did not TFO, PLH who TFO were more likely to have: >1 episode of disengagement with RTC (27% vs 14%; aRR 1.95; 1.91–1.99) and were less likely to be LTFU at the end of observation (18% vs 29%; aRR 0.62; 0.60–0.63). Among 183,151 PLH whose first VL was <50 cps/mL, those who TFO were more likely to develop a VL>50 cps/mL versus those who did not (43% vs. 34%, p<0.05). In 47,134 patients with VL<50 cps/mL at the time of TFO, 34% developed a VL>50 cps/mL at a median 31m (IQR 18–48) after transfer.

Conclusion: Transfer between PHCs occurs frequently and PLH who transfer may be vulnerable to disengagement from care and viremia, pointing to the need for intervention strategies to support the transfer process.

Figure: Engagement in care in patients on ART who do and do not transfer between PHC facilities

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**649 GEOSPATIAL RISK PROFILES PREDICT INCIDENT-HIV AREAS IN HYPERENDEMIC AFRICAN NATION**

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Background: The cause of the geographic variation in HIV epidemics across generalized hyperendemic settings is poorly understood. We assessed the role of geospatial clustering of HIV risk factors including viral load within nationally representative census enumeration areas (EAs) to predict prospectively observed incident infections in the hyperendemic setting of Eswatini.

Methods: In 2011, a household-based sample of 18,172 adults, ages 18–49 years, from 575 EAs received HIV testing and completed an administered questionnaire. All HIV-seropositive samples were tested for HIV RNA. HIV-seronegative adults were restested and reinterviewed six months later. Multi-level latent class modeling was used to identify statistically significant combinations of seven HIV risk factors (i.e., sexual activity, number of partners, casual partnerships, partner HIV status, condom use, HIV viremia at ≥20 copies/mL, and gender-age group) and to classify them into EA risk profiles of composite EA prevalences of HIV risk factors. Generalized linear regression was used to assess whether EA-level HIV seroconversion (i.e., if an EA had at least one HIV seroconversion during the follow-up period) was associated with EA risk profiles or with the observed mean EA prevalence of any single HIV risk factor across all 575 EAs.

Results: Of 11,880 HIV uninfected adults (51% male, 45% 18–24 years), 11,155 (94%) completed follow-up. Based on all risk factors, four EA risk profiles were identified, ranging from lowest (Profile A) to highest (Profile D) risk of new infections. Prevalence of EA-level HIV seroconversion increased across profiles: A (14.3%), B (21.8%), C (24.6%) and D (30.8%). EA-level HIV seroconversion...
was twofold higher in Profile D than Profile A areas (relative risk 2.13, 95% confidence interval (1.13, 4.00), p=0.02). The prevalences of unknown partner HIV status and detectable viremia in Profile D were 28% and 24%, respectively, compared to 8% and 31% in Profile A. In isolation, none of the observed mean EA prevalences of any risk factor was independently associated with EA-level HIV seroconversion.

**Conclusion:** In a generalized epidemic, a composite geospatial measure of concurrent HIV risk factors including viremia was better than viremia alone in predicting HIV incidence at an EA level. Tailoring HIV prevention and treatment interventions to area patterns of HIV risk factors may optimize the impact of national HIV response efforts in similar settings.

### Area profiles of clustered HIV risk factors and the association with area-level HIV seroconversion

<table>
<thead>
<tr>
<th>Area profile</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profile prevalence (%)</td>
<td>15</td>
<td>56</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Area prevalence of HIV risk factor (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual activity</td>
<td>94</td>
<td>84</td>
<td>91</td>
<td>85</td>
</tr>
<tr>
<td>Two or more partners</td>
<td>10</td>
<td>8</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Sexual partner</td>
<td>5</td>
<td>6</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Partner with unknown HIV status</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Never use a condom</td>
<td>42</td>
<td>39</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>HIV viremia (≥25 copies/mL)</td>
<td>31</td>
<td>21</td>
<td>27</td>
<td>24</td>
</tr>
</tbody>
</table>

**Relative risk of area-level HIV seroconversion**

- **Profile A:** 1.51 (1.40-1.63) 1.35 (1.20-1.51) 2.13 (1.94-2.44)

**Methods:** Using the igraph package in R, we first reconstructed the venue-level co-affiliation network comprised of venues reported by ≥2 MSM/TW. Then, we condensed the network to a core subset of highly densely connected venues (e.g. Hotel-1, Sauna-1, Plaza-1) with high centrality (superimposed with venue-level HIV positivity) (Panel B) identifies a co-affiliation network (Panel A) in conjunction with ordered ranking of network centrality (superimposed with venue-level HIV positivity) (Panel B) identifies a densely connected subset of venues (e.g. Hotel-1, Sauna-1, Plaza-1) with high venue-level HIV positivity.

**Conclusion:** Sexual affiliation network analysis revealed a core subset of highly connected venues in Lima that may be ideal sites at which to reach MSM/TW in high-risk sexual networks. Our results highlight a potentially valuable role for sexual affiliation network analysis as a tool to aid public health systems in effectively targeting HIV testing and prevention interventions to interrupt HIV transmission.
DYNAMICS OF HIV TRANSMISSION BETWEEN HIGH-RISK POPULATIONS IN Tijuana

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Background: Tijuana, Mexico is situated on the Mexico-US border and home to many individuals at high HIV risk. We characterized the dynamics of HIV transmission between high-risk populations in Tijuana to inform the development of targeted interventions in this binational context.

Methods: Using a comprehensive data set of HIV-1 B partial pol sequences sampled in Tijuana between 2011 and 2020 from (1) Proyecto Enlaces (PE, N=232 men who have sex with men or transgender women (MSM or TGW) and TGW living with HIV; N=187 with HIV-1 pol data) and (2) Proyecto El Cuete (ECIV, N=942 cisgender women [CW] and cisgender men [CM] who report injection drug use [IDU]; N=68 with HIV-1 pol data), we applied the following multistep phylogenetic approach: (1) maximum likelihood phylogenetic inference to identify well-supported monophyletic clades from PE/ECIV (i.e., putative transmission clusters); (2) all clades identified in step 1 were used to perform a discrete phylogeographic inference to evaluate historical introductions of HIV into Tijuana and transmission dynamics between risk groups defined based on participants’ IDU in the past month, gender identity, and the gender identity of their recent sex partners (i.e., CW and CM; MSM and TGW who did and did not report IDU).

Results: Our analysis (N=255) included sequences from 166 (65.1%) MSM, 21 (8.2%) TGW, 37 (14.5%) CM, and 31 (12.2%) CW, with 67 (33.6%) and 93 (35.9%) reporting recent IDU and transactional sex, respectively. After combining these with 107,953 publicly available HIV-1 pol sequences, we identified 15 supported clades of size ≥3 (N=88 sequences) and 39 introductions into the local epidemic (most recent common ancestor=2008.6 [95%I: 1987.6-2015.9], Fig. 1A). Discrete phylogeographic analysis of the identified clades revealed high levels of transmission events from CW and CM reporting IDU toward MSM reporting IDU (50% of all supported transmission events), between TGW who did and did not report recent IDU (36%), and from MSM toward TGW both of whom did not report recent IDU (20%) (Fig. 1B). Overall, 32% of transmission events originated from people who reported transactional sex.

Conclusion: Our findings suggest an important role of IDU in the bridging of HIV transmission across high-risk populations in Tijuana. Efforts to decrease HIV transmission among those reporting IDU and sex work may confer HIV prevention benefits across high-risk populations in Tijuana and the broader Mexico-US border region.
TRENDS AND DISPARITIES IN ACHIEVING VIRAL SUPPRESSION IN THE UNITED STATES, 2012-2018

Elizabeth Humes1, Jennifer Lee1, Jun Li2, David B. Hanna1, Vincent Marconi3, Jonathan Colasanti4, Heidi Crane4, Mari M. Kitahata5, Ronald J. Bosch6, Santita Shah7, Michael J. Silverberg8, Keri N. Althoff9, Richard Moore2, Kate Buchacz2, for the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA

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Background: Treating people with HIV (PWH) rapidly and effectively to achieve viral suppression (VS) is a key strategy in the US for ending the HIV Epidemic (EHE) Initiative. VS is critical for PWH to achieve optimal health outcomes and reduce the likelihood of drug resistance and further HIV transmission. We assessed initial VS 6 months after antiretroviral therapy (ART) initiation and characterized PWH who did not achieve this outcome.

Methods: We analyzed data on ART-naïve PWH ≥18 years of age who newly presented to care and initiated ART between 1/1/2012-6/30/2018 from 13 NA-ACCORD clinical cohorts. PWH with a clinical AIDS diagnosis >30 days prior to care entry or viral load (VL) <50 copies/mL prior to care entry were excluded. Patients were followed from ART initiation until initial VS (1 VL <200 copies/mL), death, loss to follow-up, or 6 months after ART initiation. The cumulative incidence of initial VS 6 months after ART initiation was calculated using the Kaplan–Meier estimator and stratified by calendar year. Adjusted hazard ratios (aHR) for initial VS 6 months after ART initiation were calculated using discrete time-to-event models that included the following variables at ART initiation: age, sex and HIV acquisition group, race/ethnicity, CD4 count and VL, mental health diagnoses, alcohol or drug dependence/abuse, geographic region of residence, AIDS diagnosis, ART regimen, and year of ART initiation.

Results: Among 9,807 PWH initiating ART the cumulative incidence of achieving initial VS 6 months after ART initiation increased from 77% in 2012-2013 to 83% in 2016-2018. In multivariable analysis, factors at ART initiation significantly associated with lower rates of initial VS included age <50 years, Black race, Northeast and South geographic region, lower CD4 count, a drug dependence/abuse diagnosis, injection drug user (IDU) transmission risk, initiating on a regimen that did not contain an integrase inhibitor ( INSTI), and higher VL (Figure).

Conclusion: The cumulative incidence of initial VS increased over time, suggesting increased effectiveness of ART and progress towards EHE goals. Despite these gains, we identified several groups that remain less likely to achieve initial VS within 6 months. PWH of younger age, of Black race, or with a history of drug dependence/abuse or IDU transmission risk may benefit from additional HIV care and programming support to achieve VS.

HIV CARE OUTCOMES AMONG AMERICAN INDIAN/ALASKA NATIVES IN THE UNITED STATES, 2018

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Background: In 2019, the U.S. government launched the Ending the HIV Epidemic (EHE) initiative with the aim of reducing the number of new HIV infections in the U.S. by 75%, and linking those with HIV to care and ensuring HIV viral suppression (VS) across all racial and ethnic groups to 95% by 2025. The Indian Health Service (IHS) provides federal health services to American Indian/Alaska Natives (AI/AN) who are members of the 574 federally recognized tribes consisting of 12 geographic IHS areas across the U.S. IHS is charged with fulfilling the goals of the EHE. In this analysis, we looked at differences in HIV care outcomes among non-Hispanic AI/AN compared to non-Hispanic whites based on EHE targets.

Methods: Using data reported to the National HIV Surveillance System (NHSS) through December 2019, we determined HIV care outcomes in 2018, for all AI/AN aged ≥13 years with diagnosed HIV. Data on care outcomes were available from 41 states and the District of Columbia (DC), and analyses focused on the specific counties where IHS provides services. Linkage to HIV medical care was measured by documentation of ≥1 CD4 or viral load (VL) tests ≤1 month after HIV diagnosis in 2018. VL test result of <200 copies/mL indicates VS. Evidence of VS among those living with diagnosed HIV was measured using the most recent VL test result in 2018.

Results: Among non-Hispanic AI/AN residing in IHS areas with complete laboratory data reported to NHSS in 2018, 72 (79.1%) of the 91 cases diagnosed were linked to medical care within 1 month of diagnosis. This is higher than the national percentage for non-Hispanic AI/AN (77.9%) but lower than that for non-Hispanic whites (84.4%) in IHS areas. Evidence of VS within 6 months of diagnosis was 50.5% among non-Hispanic AI/AN in IHS areas in 2018 compared to 68.4% among non-Hispanic whites. Among all 1,361 non-Hispanic AI/AN living with diagnosed HIV in IHS areas in 2018, VS was 67.7%; this is higher than national percentage for non-Hispanic AI/AN (64.0%) but lower than for non-Hispanic whites in IHS areas (74.5%).

Conclusion: HIV care outcomes were better among the non-Hispanic AI/AN population residing and receiving care in IHS areas compared to non-Hispanic AI/AN nationally, but fell short of percentages among non-Hispanic white residents within IHS areas. Various barriers to linkage and retention in HIV care among AI/AN need to be addressed to achieve the EHE goals of 95% linkage to care and 95% viral suppression.

656 STRUCTURAL FACTORS ASSOCIATED WITH HIV CARE FOR BLACK PEOPLE WITH DIAGNOSED HIV, 2017

Joseph E. Logan1, Nicole Crepaz2, Feijun Luo1, Xueyuan Dong1, Zanetta Gant1, Allison Erdt1, Candace Girard2, Nimeshkumar Patel1, Chan Jin3, Alexandra Balaji1, Patricia Sweeney1
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Black/African American (Black) people are unequally burdened by human immunodeficiency virus (HIV) in the United States. Identifying structural social determinants of health (SDH) associated with HIV care outcomes for Black people with HIV (PWH) is critical to prevention efforts.

Viral suppression among persons 213 years alive at year-end 2018 in IHS Areas and Nationally by Select Races/Ethnicities

Non-Hispanic AI/AN Areas
Non-Hispanic Whites Areas
Non-Hispanic AI/AN Nationally
Non-Hispanic Whites Nationally

Race/Ethnicity

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Methods: We used the World Health Organization’s SDH framework and data from the National HIV Surveillance System, the U.S. Census Bureau, and the Home Mortgage Disclosure Act to examine HIV care outcomes in relation to structural SDH factors among Black PWH (n=8,520) in 42 U.S. states with complete data and laboratory reporting, aged ≥18 years, with HIV diagnosed in 2017, and alive at year-end 2018. Structural SDH factors included: exposure to racial mortgage redlining; residing in states with Medicaid expansion; and residing in states with >50% of PWH receiving Ryan White services. Outcomes included: linkage to HIV care within one month after diagnosis; and having viral suppression (<200 copies on most recent test) in 2018. Adjusted prevalence ratios (aPR) with 99% confidence intervals (99%CI) accounting for socioeconomic factors are provided.

Results: Just over half (50.8%) of Black people with HIV diagnosed in 2017 resided in areas with high poverty levels. The mean racial mortgage redlining index across census tracts was 2.0, indicating that Black versus White mortgage applicants were twice as likely to be rejected for mortgage loans (adjusting for loan amount, income, and gender). However, among this sample, there was no difference in the prevalence of either outcome between those residing (versus those not residing) in areas with racial mortgage redlining. For those residing (versus those not residing) in states with Medicaid expansion, linkage to HIV care within one month after diagnosis was more prevalent (aPR:1.06; 99%CI:1.02-1.10). For those residing (versus those not residing) in states with >50% of PWH receiving Ryan White services, having viral suppression in 2018 was more prevalent (aPR:1.06; 99%CI:1.02-1.11).

Conclusion: HIV care outcomes among Black people with HIV diagnosed in 2017 did not differ across areas with various levels of racial mortgage redlining; but, work is needed to see if redlining is concentrating Black people into low income areas thereby over-exposing them to other factors related to poor HIV care outcomes. Medicaid expansion might help PWH initiate care after diagnosis; but, ongoing services like those in the Ryan White program might be needed to achieve viral suppression.

657  **HOUSING AND HIV OUTCOMES AMONG TRANSGENDER WOMEN IN SOUTH AFRICA**

Tonia Poleat1, L Leigh Ann van der Merwe2, Allanela Coetle1, Dee Adams1, Namnat Malik1, Andrea Wirtz3


Background: Laboratory-confirmed HIV prevalence estimates among transgender women (TW) in South Africa range from 45-63% versus 20% for the general population. This analysis sought to identify intervenable factors associated with self-reported HIV infection and treatment interruptions among TW.

Methods: From May to September 2018, we recruited 214 TW in Cape Town, East London, and Johannesburg through community outreach. Each TW completed an interviewer-administered survey. We collected data on structural and psychosocial factors; HIV risk behaviors; self-reported HIV status; and outcomes. Logistic regression models tested associations between structural (homelessness, income, hunger, sex work), interpersonal (social support, physical and sexual violence), alcohol use, and individual (alcohol use, medical distress) factors and 2 outcomes: HIV status among the entire sample and inability to access antiretroviral therapy (ART) at some point in the prior 12 months among the TW living with HIV (TWLHIV).

Results: 31% (67/214) of TW reported being HIV+, and 31% (20/64) of TLWHIV reported inability to access ART at some point in the prior 12 months. We found significant bivariate associations between HIV status and violence, sex work, alcohol use, and homelessness. Sex work was also significantly associated with history of violence. In multivariable models, a history of homelessness (aOR 7.7; 95%CI: 3.1, 19.2) and sex work (aOR 5.7; 95%CI: 2.5, 13.2) were most strongly associated with HIV+ status. Among TLWHIV, violence, homelessness, and medical distrust were positively associated with inability to access ART, while sexual support was negatively associated with inability to access ART.

Conclusion: Homelessness was strongly associated with reporting HIV infection and treatment interruptions, eclipsing individual and interpersonal factors.

Ensuring access to stable housing for TW is an important structural intervention that may reduce HIV risk and improve outcomes among TLWHIV. Conversely, housing discrimination may lead to homelessness among TLWHIV. Additionally, associations between sex work, violence, and HIV highlight the need for safer working conditions, including violence prevention and access to high impact HIV prevention, for TW engaged in sex work.

658  **FOOD INSECURITY AND LOWER VIRAL SUPPRESSION IN BURMESE MIGRANTS LIVING WITH HIV**

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Background: With Thailand regarded as food secure for nearly a decade, at-risk groups including migrant workers still face food insecurity and have higher risk of acquiring HIV infection. People living with HIV and facing food insecurity have been documented to have higher risk of poor health and HIV treatment outcomes, notably altered risk behaviours and decreased adherence to antiretroviral therapy (ART). However, research on direct links between food insecurity and treatment outcomes such as viral suppression is scarce. The aim of this study was to investigate how food insecurity are associated with income, viral suppression, ART treatment and ART adherence in Burmese migrant workers living with HIV in Chiang Mai province of northern Thailand.

Methods: Data collected through face-to-face survey was combined with routine laboratory tests in a cohort of 316 migrants (113/203 M/F) living with HIV. 11 treatment centers for HIV in rural and urban Chiang Mai gathered data on ART use and adherence, physical and mental health, sexual behaviour, socio-demographics and food security (Household Food Insecurity Access Scale (HFAS-III)). Using a step-down multivariate logistic regression, we calculated odds ratios (OR) and 95% confidence intervals (CI), adjusting for confounders, including ART regimen.

Results: In this cross-sectional study, 48.7% (n=162) of migrant workers living with HIV reported food insecurity, and 14.2% (n=45) fulfilled criteria for severe food insecurity. Most respondents were ART-adherent 96.8% (n=305), and virally suppressed 93.5% (n=290), with 4.1% (n=13) expressing symptoms of clinical depression. In adjusted analysis, food insecurity was associated with lack of viral suppression [OR=4.13, CI(1.22–14.00) and perceived poverty/lack of income [OR=5.96, CI=2.58–13.76].

Conclusion: Burmese migrant workers living with HIV in Chiang Mai report high adherence to ART and are mostly virally suppressed. Food insecurity is here linked to viremia and poverty or lack of income, suggesting that a subset of migrants face multiple burdens that increase their likelihood of becoming viremic. With food insecurity and poverty rising as a result of the COVID-19 pandemic this may end up negatively impacting HIV treatment outcomes.

<table>
<thead>
<tr>
<th>Logistic regression</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
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<td></td>
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<tr>
<td>Age</td>
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<td>0.99–1.05</td>
<td>0.13</td>
</tr>
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<td>Ref: Insufficient</td>
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<td>1.14–5.46</td>
</tr>
<tr>
<td></td>
<td>Ref: Sufficient w/ savings sufficient</td>
<td>5.96</td>
<td>2.58–13.76</td>
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<td>1.33</td>
<td>0.80–2.20</td>
</tr>
<tr>
<td>FHS-9</td>
<td>Ref: No depressive symptoms</td>
<td>3.48</td>
<td>0.80–15.20</td>
</tr>
<tr>
<td>Adherence</td>
<td>Ref: &gt;95%</td>
<td>3.04</td>
<td>0.64–14.50</td>
</tr>
<tr>
<td>Viral load</td>
<td>Ref: Suppressed</td>
<td>4.13</td>
<td>1.22–14.00</td>
</tr>
</tbody>
</table>

99%CI: 1.6, 22.2.

1658 FOOD INSECURITY AND LOWER VIRAL SUPPRESSION IN BURMESE MIGRANTS LIVING WITH HIV
DISTANCE TO HIV TREATMENT CENTER AND ASSOCIATION WITH ART USE AMONG MEN IN UGANDA

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Background: Background: There is underutilization of antiretroviral therapy (ART) among HIV positive men but more pronounced among adolescent and young adults (AYA) aged 15–24 years, compared to adult men (25–49 years). We used Geo Spatial data to determine the distance to the nearest treatment center from each home of an HIV-positive male respondent. We examined the association of distance to ART initiation in a community-based cohort in south central Uganda.

Methods: Method: We analyzed data from the Rakai Community Cohort Study (RCCS) for HIV-positive men between 2016 and 2018. We used GPS data and QGIS software to estimate the distance in Coordinate Reference System (CRS) units between each place of residence and the nearest treatment center offering ART services. Ever initiated on ART was the outcome variable and distance the major predictor variable. Other covariates included: community type (fishing, trading or agrarian), marital status, religion, occupation, alcohol consumption, education, mobility and number of sexual partners. We used a Poisson GLM to establish the relative risk associated with ART initiation. ART for key populations in fishing communities was initiated using test and treat, whereas other communities initiated ART based on CD4 levels.

Results: Results: The analysis included HIV positive men (n=1,289), ever initiated on ART (n=971), never on ART (n=318). From the spatial pattern, the mean distance to health centers is 0.05 in CRS units. Distance to health centers was not statistically associated with ART initiation. The relative risk of not being on ART was increased among the youth (15-24 years) compared to older men (RR: 1.68; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men on ART was increased among the youth (15-24 years) compared to older men (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67). There was an increased risk of non-ART use among men who were either divorced or never married compared to those currently married (RR: 1.60; CI 1.00, 2.54) and (1.55; CI 1.19, 2.01), respectively. There was increased risk among residents from non-fishing communities compared to the fishing communities and the immigrants compared to the more permanent (RR: 1.69; CI 1.06, 2.67).

Conclusion: Conclusion: There is no evidence that distance to treatment center affects ART initiation among men. However, ART use among young unmarried men, immigrants and those residing outside fishing communities is low. Test and treat provided in fishing communities increased uptake of ART.

REASONS FOR NONDISCLOSURE OF HIV-POSITIVE STATUS TO HEALTH CARE PROVIDERS, MOZAMBIQUE

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1Barcelona Institute for Global Health, Barcelona, Spain, 2Manhiça Health Research Centre, Manhiça, Mozambique, 3Fundação Áril Glaser Centro de Saúde e Pesquisa, Maputo, Mozambique, 4Centers for Disease Control and Prevention, Maputo, Mozambique

Background: Background: There is underutilization of antiretroviral therapy (ART) among HIV positive status or prior testing to clinicians may lead to re-testing and, consequently, waste of scarce resources and distorted estimates of persons who know their HIV-positive status. In 2015 in Mozambique, an estimated one-third of people tested HIV positive but already knew their status. To our knowledge, our study is the first to assess the barriers that prevent people living with HIV (PLHIV) from disclosing their HIV-positive status to healthcare providers during a PICT testing campaign.

Methods: Method: This analysis was nested in a larger PICT cross-sectional study performed in the Manhiça District, southern Mozambique in 2019, in which healthcare providers actively asked patients about their HIV status. The identity of patients who tested positive was crosschecked with the hospital database to detect current or previous enrolment in care. PLHIV who did not disclose their HIV-positive status were invited to participate and responded to a questionnaire designed to explore barriers, patterns of community/family disclosure, and stigma and discrimination.

Results: Results: Our results showed that 17.3% of participants who tested positive during a PICT campaign already knew their HIV status and had enrolled in care but did not disclose it to the PICT campaign healthcare provider. Of participants, 92% reported previous mistreatment by general healthcare providers as a reason for nondisclosure during PICT (Figure). Other reasons included the desire to confirm if they were cured (33.3%) or to re-engage in care (23.5%). Among respondents, 83.9% reported having disclosed their HIV status within their close community, 72.2% reported being victims of verbal or physical discrimination, and 46.7% reported that their HIV status affected their daily activities.

Conclusion: Previous mistreatment by healthcare workers was the main barrier to disclosing HIV status. The high proportion of those disclosing their HIV status to their community but not to healthcare providers suggests that challenges with patient-provider relationships affect this care behavior rather than general stigma and discrimination. Improving patient-provider relationships could increase trust in healthcare providers, reduce nondisclosures, and help optimize resources and accurate estimates of PLHIV aware of their HIV-positive status.

DEPRESSION AND HIGHER EDUCATION ARE DRIVERS OF HIV STIGMA IN A SOUTH AFRICAN TOWNSHIP

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1University of Washington, Seattle, WA, USA, 2AIDS Healthcare Foundation, Durban, South Africa, 3University of KwaZulu-Natal, Durban, South Africa

Background: HIV stigma remains a significant barrier to HIV testing and engagement in care. Despite expanded access to HIV treatment, HIV-related stigma has not declined in LMICs. We measured the prevalence of HIV-related stigma, and identified sociodemographic and clinical factors associated with
stigmatizing attitudes (negative attitudes towards individuals who may be HIV positive) and anticipated stigma (the expectation of experiencing prejudice and stigmatizing behaviors if the respondent became HIV positive).

Methods: We conducted a cohort study of adults seeking HIV testing between 2013 and 2018 in Umlazi township, South Africa. We used a validated HIV stigma scale consisting of seven questions about stigmatizing attitudes and five questions about anticipated stigma and obtained survey responses prior to HIV testing. We coded responses to each question 0—2 (0=no stigma, 1=mild stigma, 2=high stigma) to calculate on overall stigma score and categorized participants as either having “no stigma” or “mild or high stigma”.

We used multivariate logistic regression to identify statistically significant sociodemographic and clinical correlates of holding stigmatizing attitudes or having anticipated stigma.

Results: 7,724 (98.0%) of 7,877 participants enrolled completed the 12-question stigma scale. 1,318 (16.9%) reported at least one stigmatizing attitude and 2,396 (30.8%) reported at least one anticipation of stigma. In separate adjusted multivariate models, the strongest predictors of having both stigmatizing attitudes and anticipated stigma were having depressive symptoms (“stigmatizing attitudes” adjusted odds ratio (aOR) = 19.49 and “anticipated stigma” aOR = 9.34) and having attended University (“stigmatizing attitudes” aOR = 6.89 and “anticipated stigma” aOR = 7.00) (Table). Additional significant predictors of HIV-stigma included employment, female sex, younger age, single status, not attending church, and having a partner that had not tested for HIV or had tested negative for HIV.

Conclusion: In an urban township of South Africa, both stigmatizing attitudes and perceived stigma continue to be common. Having attended university and having depressive symptoms appear to be important risk factors for holding stigmatizing attitudes and for anticipating HIV stigma against oneself. Addressing HIV-stigma among those who may need greater support following a diagnosis of HIV may be important for improving HIV treatment outcomes.

<table>
<thead>
<tr>
<th>Socioeconomic Characteristics</th>
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<th>Anticipated stigma [N=3,896]</th>
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<td>Sex</td>
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<tr>
<td>Female</td>
<td>1.0 (95% CI 1.0–1.0)</td>
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<td>1.0 (95% CI 1.0–1.0)</td>
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<td>≥18</td>
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<tr>
<td>Education</td>
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<tr>
<td>≤12th</td>
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<td>&gt;12th</td>
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<td>Depressive Symptoms</td>
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<td>9.64 (95% CI 7.98–11.06)</td>
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<td>1.52 (95% CI 1.39–1.64)</td>
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<td>Partner Tested for HIV</td>
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<tr>
<td>Yes</td>
<td>1.16 (95% CI 1.10–1.23)</td>
<td>0.927 (95% CI 0.87–1.00)</td>
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</table>

Note: aOR=adjusted odds ratio; CI=confidence interval. The adjusted model for stigmatizing attitudes also included income, HIV status, and a traditional healer within the last six months. The adjusted model for anticipated stigma also included HIV status and children.

662 THE TREATMENT GAP FOR MENTAL DISORDERS IN PEOPLE LIVING WITH HIV IN SOUTH AFRICA


Background: Mental disorders are common in people living with HIV (PLWH) but often remain untreated. This study aimed to explore the gap in access to mental health services between these programs in terms of access to mental health services.

Methods: We conducted a cohort study using linked pharmacy and hospitalization data from one private care, two public primary care, and one public tertiary care ART programs in South Africa. We restricted our study to adults aged 15–49 years. Patients were followed-up from January 1, 2012 to December 31, 2017. We estimated the proportion of patients treated for a mental disorder (pharmacological or inpatient) in the ART programs in each calendar year, and examined factors associated with the rate of treatment for mental disorders in those programs. We calculated the treatment gap for mental disorders as the discrepancy between the 12-month prevalence of mental disorders in PLWH in South Africa (based on data from the Global Burden of Disease study) and the proportion of patients treated for a mental disorder in the ART programs in each calendar year. We calculated adjusted rate ratios (aRR) for factors associated with the rate of treatment of mental disorders using Poisson regression.

Results: 182,285 ART patients were followed-up over 405,153 person-years. In 2017, the estimated treatment gap for mental disorders was 40.5% (95% CI 19.5%–52.9%) for patients followed-up in private care, 96.5% (95% CI 95.0%–97.5%) for patients followed-up in public primary care, and 65.0% (95% CI 36.5%–85.1%) for patients followed-up in public tertiary care ART programs (Figure). Rates of treatment with antidepressants, anxiolytics and antipsychotics were 17 (aRR 0.06, 95% CI 0.06–0.07), 50 (aRR 0.02.95% CI 0.01–0.03), and 2.6 (aRR 0.39, 95% CI 0.35–0.43) times lower in public primary care programs than in the private sector ART program.

Conclusion: There is a large treatment gap for mental disorders in PLWH in South Africa. We found substantial disparities in access to mental health service between patients receiving ART in the public vs. the private sector. In the public sector and especially in public primary care, common mental disorders remain largely untreated in PLWH.


663 HEALTH DISPARITIES DUE TO NONADHERENCE TO ANTIPSYCHOTICS IN PLWH WITH SCHIZOPHRENIA

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British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada; St Paul’s Hospital, Vancouver, Canada, 3University of California San Diego, San Diego, CA, USA, 4University of British Columbia, Vancouver, Canada

Background: Non-adherence to antipsychotics is the greatest obstacle to treating schizophrenia, and it is likely to exacerbate the clinical implications of HIV and its treatment. We assessed the economic and clinical impact of non-adherence to antipsychotics among people living with HIV/AIDS (PLWH) and schizophrenia in British Columbia, Canada.

Methods: Eligible individuals were in the STOP HIV/AIDS cohort during 2001-2016, diagnosed with schizophrenia, on antipsychotics for ≥ 1 year, and followed for ≥1 year from schizophrenia diagnosis date or January 1, 2001, whichever occurred last. Adherence to antipsychotics was measured using the proportion of days covered methodology. A Two-Part model assessed the marginal effect of adherence on healthcare costs (in 2016 CAD), including hospitalizations, physician visits and medication dispensations. Logistic regression or generalized linear mixed models examined the effect on virologic failure (viral load >200 copies/mL), hospital readmissions within 30 days, and length of hospital stay. Models were adjusted for several confounders.

Results: Among 726 PLWH with schizophrenia, ≥80% adherence to antipsychotics increased from 34% in 2001 to 55% in 2016. We observed no difference in adherence to antipsychotics among those who used only
injectable form, only non-injectable form, and a combination of both. Similarly, no difference in adherence was observed among individuals who have ever consumed typical first-generation antipsychotics and those who consumed only atypical/second-generation antipsychotics. We observed that individuals with poor adherence to antipsychotics were also poorly adhered to antiretrovirals, especially in the earlier study years. Compared to adherent individuals, annual hospitalization and physician visit costs were higher among non-adherent individuals ($5300), particularly among women ($3386) and people who ever injected drugs ($5102) (Figure 1). Non-adherent individuals also experienced higher virologic failure (adjusted Odds Ratio [aOR]=1.65, 95% CI: 1.06-2.56), more hospital readmissions (aOR=1.52, 95%CI: 1.29-1.79), and longer hospital stays (adjusted Mean Ratio=1.51, 95%CI:1.39-1.64).

Conclusion: Our results showed that implementing strategies and interventions to increase antipsychotic adherence, focusing on women and people who have ever injected drugs, will be critical in addressing this public health challenge.

Figure 1. Marginal effect on annual healthcare costs among non-adherent PLWH with schizophrenia compared to their adherent counterparts in our study population, overall and stratified by (A) Gender and (B) People who have ever injected drugs (PWID) status (adjusted to 2016 CAD).

664 OPIOID USE DISORDER, AGONIST THERAPY, AND MORTALITY AMONG PEOPLE LIVING WITH HIV

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1British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada
2University of Washington, Seattle, WA, USA
3British Columbia’s (BCs) overdose crisis has had devastating impacts on the general population, but little is known on how it has affected People living with HIV (PLWH). We therefore characterized the annual crude mortality rates for overdose-related deaths, and examined potential risk and protective factors for overdose-related mortality in our population.

Methods: Using the BC STOP HIV/AIDS database, we examined overdose-related deaths among PLWH in BC, Canada who were followed for >6 months between April 1, 2009–March 31, 2017 in BC, Canada. Overdose-related deaths were ascertained using ICD-10 codes in the Vital Statistics registry. Confounder-adjusted logistic regression models examined whether opioid use disorder (OUD) diagnoses (defined as an ever-recorded OAT dispensation, ever ≥ 3 physician claims, or 1 hospital admission) and opioid agonist therapy (OAT) prescriptions were associated with overdose-related mortality.

Results: Of 9594 PLWH in BC during the study period, 166 (18%) died of overdose-related causes. The crude period mortality rate was 2.89 deaths per 1,000 person-years (PY), with women experiencing 1.81 times higher rates of overdose-related mortality. Annual mortality rates began increasing in 2012/13 to a high of 5.1 deaths per 1,000 PY in 2016/17. Of those who died from overdose, 75% met the prescription criteria for OAT, while 51% did not receive an OAT prescription. Women were significantly more likely to be prescribed neuropharmacologic pain relievers (NPRs), selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), and sedatives. After adjustment for health authority, gender, HCV co-infection status, diagnosis of a mental health illness, date of most recent OAT prescription, and age at death, people who were diagnosed with OUD had 3.48x the odds of dying of overdose compared to all causes of death, while the absence of OAT was non-significantly associated with risk of overdose deaths (AOR: 1.09, 95% CI: 0.70-1.7).

Conclusion: Overdose-related mortality among PLWH, which has been increasing amid the opioid crisis, is significantly correlated to chronic prescription trends and OUD, and non-significantly associated with the absence of OAT. Further research into these factors, particularly as mediated by OAT, may shed light on vulnerabilities related to prescription practices, drug interactions with OAT medications, and missed opportunities for OAT.

665 SEXUAL HEALTH SERVICES FOR PEOPLE WHO ENGAGE IN EXCHANGE SEX: NEEDS AND OPPORTUNITIES

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1University of Washington, Seattle, WA, USA

Background: People who exchange sex (PWES) for money or drugs are at increased risk of HIV and other sexually transmitted infections (STIs) and may need tailored prevention and care services. Our objective was to characterize patients in the Public Health - Seattle & King County (PHSKC) - Sexual Health Clinic who reported engaging in exchange sex and identify opportunities for improved service.

Methods: We conducted a cross-sectional analysis of patient encounters for new problem visits October 2010 - March 2020 with a completed computer assisted self-interview including sex assigned at birth, gender identity, and receipt of money or drugs for sex, ever or in the past year. Visits were the unit of analysis. We analyzed demographics, STI and HIV history, Hepatitis C (HCV) testing and treatment history, PrEP use, and reason for visit among encounters in which the patient reported ever exchanging sex, stratified by gender. Our analysis focused on people who reported a lifetime history of exchange sex because the characteristics of this group did not differ substantially from people who reported exchanging sex in the past year. We compared characteristics of PWES ever vs never using chi square tests.

Results: During the study period 30,327 unique patients attended 64,680 clinic visits. At these visits, 1,470 (2%) reported exchange sex in the past 12 months and 3,097 (5%) reported ever exchanging sex, among whom 1,943 (63%) were cis-men, 969 (31%) were cis-women, and 185 (6%) were transgender persons. Among PWES, the most common reason for coming to clinic was wanting a STI test (61%), HIV test (45%), or STI symptoms (40%). Compared to patients who never exchanged sex, PWES were more likely to report homelessness, injection drug use (IDU), STIs in the past 12 months, and a prior diagnosis of HIV or HCV (Table 1). Among PWES, homelessness was more common among cis-women (33%) compared to cis-men (21%) and transgender persons (18%), and IDU was higher among cis-men (42%), than cis-women (33%), and transgender persons (29%). More cis-men reported >5 sexual partners (53%) or an STI (51%) in the past year compared to cis-women (16% and 26%, respectively). Among PWES who did not have a prior HIV diagnosis, 17% were on PrEP (22% of cis-men, 1% of cis-women, 43% of transgender).

Conclusion: Many PWES in the Sexual Health Clinic had complex barriers to care including homelessness and IDU, and higher percentage of STIs, HIV, and HCV. Clinic visits are an opportunity to increase PrEP use and HCV treatment for PWES.
666 METHAMPHETAMINE USE AND HIV/STI RISK AMONG USERS OF SUBSTANCE USE TREATMENT PROGRAMS

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3 University of Nebraska Medical Center, Omaha, NE, USA, Tulane National Primate Research Center, Covington, LA, USA

Background: In recent years, methamphetamine use has been on the rise in the United States. At the same time, the rates of many sexually transmitted infections (STIs) have risen sharply, including gonorrhea, chlamydia, and syphilis. Further, in this analysis, we sought to ascertain whether the risk of STIs and HIV among methamphetamine users differs on the basis of participation in substance use treatment programs.

Methods: Data came from the nationally representative, public dataset, the National Survey on Drug Use and Health (NSDUH), 2015-2019. Among adult participants, survey-weighted logistic regression analyses were used to assess the relationship between past year methamphetamine use and risk of HIV and STIs. Analyses were stratified by methamphetamine treatment utilization during the past year and adjusted for demographic and other risk factors.

Results: Among participants in the analytic sample (N = 207,913), 2,034 (1.0%) reported past year methamphetamine use, 599 (0.3%) reported receiving treatment for its use, 6,158 (3.0%) tested positive for any STI in the past year, and 419 (0.2%) for HIV ever in their lifetime. Weighted estimates produced 1.74 million methamphetamine users per year, 510,542 who sought treatment for methamphetamine use in the past year, 5.22 million with any STI in the past year, and 353,675 with HIV in their lifetime. In analyses stratified by treatment use, past year methamphetamine use was associated with increased risk of STIs among those who did not receive treatment (adjusted odds ratio [aOR] = 3.63; 95% confidence interval [CI]: 2.71, 4.85) and among those who did receive treatment (aOR = 2.20; 95% CI: 1.02, 4.72). Regarding HIV, past year methamphetamine use was associated with increased risk of infection among those not receiving treatment (aOR = 8.64; 95% CI: 4.99, 14.96) while no significant difference in risk based on methamphetamine use was observed among those who did receive treatment in the past year.

Conclusion: In this analysis, we demonstrated a strong relationship between methamphetamine use and risk of HIV and STIs that differed based on receipt of treatment for methamphetamine use. Notably, the risk of STIs associated with methamphetamine use was lower among those who received treatment for methamphetamine use compared to those who did not. These findings suggest that integrated STI, HIV, and substance use treatment programs may yield substantial public health benefits.

667 CANNABIS USE ASSOCIATED WITH DECREASED ART ADHERENCE IN AGING PEOPLE WITH HIV

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1 Tulane National Primate Research Center, Covington, LA, USA; 2 Harvard TH Chan School of Public Health, Boston, MA, USA; 3 University of California San Diego, La Jolla, CA, USA; 4 University of Nebraska Medical Center, Omaha, NE, USA; 5 Albert Einstein College of Medicine, Bronx, NY, USA; 6 University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Background: Cannabis is commonly used in the United States, particularly among people with HIV (PWH). Conflicting evidence exists on the impact of cannabis use on care of PWH, particularly among older adults. Here, we leveraged data collected through ACTG A5322, an observational study of PWH ≥40 years in long-term follow-up, to longitudinally characterize associations between cannabis use and antiretroviral therapy (ART) adherence.

Methods: A5322 participants with ≥2 substance use surveys were included. At each visit, participants were categorized as 100% ART adherent if they indicated no missed doses of antiretroviral medication within the past 7 days, and as <100% adherent otherwise; visits during which participants were not on ART were excluded. Cannabis use was updated at each visit; participants were categorized as current cannabis users if they reported use within the past month, intermittent users if they reported use within the past year but >1 month ago, and non-users if they reported use ≥1 year ago or never.

Table 1: Characteristics of patient visits attending the PHHSK Sexual Health Clinic

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n; [95% CI])</th>
<th>Never exchanged sex (n; %)</th>
<th>Exchange sex over 3.097 (n; %)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homeless, past 12 months</td>
<td>3,570 (5)</td>
<td>2,325 (4)</td>
<td>3,555 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IDU, past 12 months</td>
<td>516 (8)</td>
<td>429 (8)</td>
<td>512 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>STI diagnosis, past 12 months</td>
<td>1,657 (3)</td>
<td>1,249 (2)</td>
<td>1,657 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior ART diagnosis</td>
<td>4,094 (6)</td>
<td>3,655 (6)</td>
<td>4,094 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior ART adherence</td>
<td>18,021 (30)</td>
<td>14,009 (30)</td>
<td>18,021 (30)</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Results: Among patients with ≥2 substance use surveys were included.

Conclusion: Further research is needed to elucidate the underlying biobehavioral mechanisms by which cannabis use elicits decreased ART adherence in older PWH and the downstream impacts on risk for comorbid conditions.

Table: Adjusted association between cannabis use and ART non-adherence

<table>
<thead>
<tr>
<th>Cannabis use group</th>
<th>Unadjusted Model</th>
<th>Weighted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current user (vs non-user)</td>
<td>1.05 (1.00, 1.10)</td>
<td>1.05 (1.00, 1.10)</td>
</tr>
<tr>
<td>Current user (vs intermittent)</td>
<td>1.43 (1.07, 1.91)</td>
<td>1.07 (1.01, 1.81)</td>
</tr>
<tr>
<td>Intermittent (vs non-user)</td>
<td>1.30 (0.88, 1.91)</td>
<td>0.94 (0.61, 1.57)</td>
</tr>
</tbody>
</table>

*Stabilized Inverse probability weights adjusting for drop-out, static covariates (sex, race/ethnicity, education level, smoking status, years on ART), and time-varying covariates (Alcohol use, non-cannabis substance use, physical/mental activity over)

668 A WEB TOOL TO PROJECT LOCAL IMPACT OF INTERVENTIONS TO END HIV EPIDEMICS IN THE US

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Background: The Ending the HIV Epidemic (EHE) Initiative aims to reduce HIV incidence by 90% by 2030 through a combination of rapidly diagnosing new HIV infections, preventing infection in susceptible populations through interventions such as pre-exposure prophylaxis (PrEP), and treating people with HIV (PWH) to achieve viral suppression. HIV epidemics are localized in nature, and the levels of testing, PrEP, and viral suppression needed to achieve EHE goals in specific locations are unclear. A user-friendly, location-specific tool to address this question could aid HIV decision-makers in choosing pathways to reach EHE goals.

Methods: The Johns Hopkins Epidemiological and Economic Model (JHEEM) is a dynamic, compartmental model representing the HIV epidemic, calibrated to 32 Metropolitan Statistical Areas (MSAs) which encompass the 48 high-burden counties plus Washington DC identified by the EHE plan. The model makes projections of incidence, prevalence, reported diagnoses, and mortality for interventions that target multiple demographic strata and combine yearly HIV testing, PrEP, and viral suppression. The interactive web tool allows users to visualize model projections for potential interventions in any of the 32 MSAs. Additionally, users can design interventions combining different levels of HIV testing, PrEP, and viral suppression, and run simulations to evaluate these interventions in real time.

Results: The web tool is publicly available at www.jheem.org, with data visualizations as shown in the Figure. In order to achieve the EHE goal of reducing incidence by 90% over a decade, most MSAs are projected to require interventions that target multiple demographic strata and combine yearly HIV
testing, PrEP uptake of 25-50% in at-risk individuals, and viral suppression of 80-90% across key populations.

**Conclusion:** The EHE goal of 90% reduction in HIV incidence is achievable, but will require substantial improvements in HIV testing, PrEP, and viral suppression among PWH. Our publicly available web tool allows health officials to project which interventions are most likely to achieve EHE goals in their local environment.

**Results:** Estimates of the 10-year tPAF (Fig.C) were consistently greater than the 1-year tPAF (Fig.B), which was greater than the % HIV infections transmitted in 1 year for each KP (Fig.A), reflecting increasing numbers of secondary transmissions captured by each indicator. For FSW, their clients and MSM, the acquisition indicator was consistently lower than the three transmission indicators. Whereas for young women, the acquisition indicator tended to be larger, suggesting that they acquire more infections than they transmit. Agreement on the greatest KP contributor across all indicators was rare (3 models), even for related indicators related to % incident infections acquired/transmitted (4 models), but more common for the 1-10-year tPAFs (13 models).

**Conclusion:** Using one indicator is insufficient to fully characterize the contribution of KP resulting from HIV prevention/treatment gaps in the settings explored. Distributions of HIV acquisition and longer-term tPAF appear to most appropriately reflect the relative contribution of different KP across diverse African HIV epidemics.

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**MODEL-BASED COMPARISON OF THE CONTRIBUTION OF KEY POPULATIONS TO HIV EPIDEMICS**

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**Background:** Comparing the contribution of key populations (KP) including female sex workers (FSW), their clients, men who have sex with men (MSM) and young women (15-24 years old) to HIV epidemics is important to plan effective intervention programs. We used mathematical models to compare four commonly used indicators.

**Methods:** Fourteen transmission-dynamic mathematical models of HIV, calibrated to different African settings, reflecting existing levels of intervention/treatment and existing gaps, were used to derive four indicators for each KP: (1) % incident infections acquired in 2020, (2) % incident infections transmitted in 2020, (3) transmission population attributable factor (tPAF) over 1-year (2020), (4) tPAF over 10 years (2010-2020). KP-specific correlation plots were used to compare indicators and to assess the level of agreement between indicators on the greatest contributor to HIV.

**Results:** The contributions of population subgroups to HIV epidemics were explored. Distributions of HIV acquisition and longer-term tPAF appear to most appropriately reflect the relative contribution of different KP across diverse African HIV epidemics.

**Conclusion:** Using one indicator is insufficient to fully characterize the contribution of KP resulting from HIV prevention/treatment gaps in the settings explored. Distributions of HIV acquisition and longer-term tPAF appear to most appropriately reflect the relative contribution of different KP across diverse African HIV epidemics.

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**AGING AND THE HIV EPIDEMIC AMONG MSM IN THE US: A COMPARISON OF 2 SIMULATION MODELS**

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**Background:** With increasing life expectancy, more MSM on ART are likely to survive past 65y and will be at risk for multimorbidity. Using two independent, individual-based models of HIV, we compared projections of the age and number of MSM on ART in the US from 2020 to 2030.

**Methods:** We populated the CEPAC microsimulation model with CDC data to project the HIV epidemic among MSM from 2014 (mean±SD age, 45.2±12.3 years) to 2030 (assumed 82% diagnosed, 66% in care and on ART, 54% virally suppressed). We estimated age- and/or CD4-stratified mortality for MSM on/off ART but did not stratify by race. We used model-projected viral load distributions to estimate annual incident HIV cases from 2015-2030 (mean age at infection, 33.4±11.0 years), assuming 40% PrEP coverage with 62% adherence after 2016.

**Results:** In the PEARL agent-based simulation of people on ART in the US, we applied observed parameter values from N A-ACCORD for the age and CD4-distribution of MSM assuming 40% diagnosed, 66% in care and on ART, and 54% virally suppressed. We estimated age- and/or CD4-stratified mortality for MSM on/off ART but did not stratify by race. We used model-projected viral load distributions to estimate annual incident HIV cases from 2015-2030 (mean age at infection, 33.4±11.0 years), assuming 40% PrEP coverage with 62% adherence after 2016.

**Conclusion:** Using one indicator is insufficient to fully characterize the contribution of KP resulting from HIV prevention/treatment gaps in the settings explored. Distributions of HIV acquisition and longer-term tPAF appear to most appropriately reflect the relative contribution of different KP across diverse African HIV epidemics.
671 CALIBRATION OF THE VACS INDEX 2.0 FOR ESTIMATING MORTALITY AMONG PWH IN NORTH AMERICA

Kathleen A. McGinniss, Amy Justice, Richard Moore, Michael J. Silverberg, Keri N. Althoff, Maile Karris, Viviane D. Lima, Heidi Crane, Michael A. Horberg, Marina B. Klein, Stephen Gange, Kelly Gebo, Angel Mayor, Janet P. Tate, for the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of iDEA

Background: The Veterans Aging Cohort Study (VACS) Index 1.0 incorporated general and HIV-specific health biomarkers (age, hemoglobin, FIB-4, eGFR, Hepatitis C virus [HCV], HIV-1 RNA, and CD4) and discriminated mortality risk in persons with HIV (PW). Conversion from categorical to continuous forms of variables and the addition of BMI, total white blood count, and albumin (VACS Index 2.0) substantially improved discrimination. In preparation for estimating remaining life expectancy, our current aim is to evaluate the calibration of VACS Index 2.0 in the North American AIDS Cohort Collaboration (NA-ACCORD), which comprises over 20 cohorts in the US and Canada, including VACS.

Methods: We included VACS and three other NA-ACCORD cohorts that collected the required data elements and ascertained mortality with the National Death Index. Among participants on ART for at least one year, we randomly chose a VACS Index 2.0 at least one year after start of ART as “baseline” (ranging from 10/1999 – 4/2018) to ensure the sample included PWH at different stages of HIV.

Results: Of 6,429 PWH in the three NA-ACCORD cohorts and 30,919 in VACS, median age was 46 and 56 years; 20% and 3% were women; 38% and 48% were African-American, 40% and 4% White; and 24% and 22% had a history of HCV, respectively. Median follow-up time was 3.5 years (1.9-5.0). VACS Index 2.0 showed greater discrimination than VACS Index 1.0 overall (0.80 vs. 0.77, p<.001) and for all subgroups. In overall and subgroup analyses, predicted and observed mortality largely overlapped, although was overestimated among women and those less than 50 years.

Conclusion: VACS Index 2.0 improves discrimination over VACS Index 1.0 in NA-ACCORD and across a wide range of subgroups. Calibration is good overall although there is overestimation in certain subgroups.

672 PREDICTIVE MODELS OF ART RESPONSES AMONG ACUTELY INFECTED INDIVIDUALS


Background: The present study employed a combination of data science and inferential analytic methods to delineate the early and ongoing risk factors for clinical phenotype variability in a large group of individuals who initiated treatment during acute HIV (AHI).

Methods: Participants included 412 Thai adults enrolled in RV254/SEARCH 010 who completed longitudinal multi-omic (e.g., viral, immune, neuro, psychosocial) assessments before and after 144 weeks of ART. Individuals were classified into favorable vs. unfavorable clinical phenotypes at weeks 96 and 144 using previously established criteria (CD4 T cell count >500, CD4/CD8 T cell ratio >1.0, and presence of viral blips). Outcomes included phenotype designation, baseline predictors, and risk trajectories modeled through weeks 96 and 144.
post ART. Group comparisons and hierarchical clustering examined correlates of trajectory subgroup membership.

**Results**: Participants were mostly male (97%), Fiebig stages I-III (86%), with a CD4 T cell count >350 at baseline and undetectable viral status by week 24. Less than half (40% and 41%) of the study sample achieved a favorable clinical phenotype at weeks 96 and 144, respectively. Baseline CD4/CD8 T cell ratio was the strongest determinant of clinical phenotype designation at both follow-up time points. Older age, increased self-report of depressive symptoms, and increased neutrophil count at baseline contributed to the prediction models. Multivariate trajectory analysis revealed five subgroups of CD4, CD8 and CD4/CD8 ratio profiles (see Figure 1). Two of the subgroups (46% of the sample) exhibited early and chronic CD4/CD8 T cell ratio inversion, owing to either incomplete CD4 T cell recovery (max CD4 <500; 36% of the sample) or persistently increased CD8 T cells in the context of robust CD4 recovery (max CD4 >850). Baseline depressive symptoms, later Fiebig stage at ART onset, and levels of IL-7, IL-23, C227, Tim-3, and RANTES differentiated the CD4/CD8 T cell ratio trajectory subgroups and clinical phenotype designation at week 144.

**Conclusion**: A combination of psychosocial, viral, and immune factors (including myeloid and lymphocyte T cell populations) at the time of ART onset predict clinical phenotype variability after 144 weeks of treatment. Baseline risk variables differentiate CD4 and CD6 subgroups and chronic CD4/CD8 T cell ratio inversion and clinical phenotype designation.

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**Figure 1**: Trajectories of CD4, CD8 and CD4/CD8 T cell ratio from baseline through week 144.

![Figure 1](image)

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**673 DEVELOPING A PREDICTIVE MODEL FOR HIV CLINICAL CARE DISENGAGEMENT**

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¹Duke University, Durham, NC, USA

**Background**: Disengaging from care is associated with all-cause mortality in people living with HIV (PLWH); therefore, tools that can assist with the early identification of persons at-risk for falling out of care are needed. The electronic health record (EHR) serves as an invaluable platform to create such tools. We present an EHR-based predictive model for early identification of persons at high-risk of falling out of HIV care.

**Methods**: We analyzed EHR data from HIV clinic patients in care at Duke between Jan 2014 and Dec 2018. Clinical data on 38 candidate variables (demographics, sexually transmitted disease (STD) diagnoses, substance abuse, mental health and healthcare utilization patterns) were collected for possible inclusion in the model. The outcome of interest, disengaging from care, was defined as failure to attend an HIV clinic appointment for ≥ 12 months after inclusion in the model. The outcome of interest, disengaging from care, was defined as failure to attend an HIV clinic appointment for ≥ 12 months after inclusion in the model. LASSO regression was used in selecting features for a logistic model. The model was calculated to assess model performance, and estimated coefficients were transformed to adjusted odds ratios.

**Results**: 2301 subjects (mean age 47 years; 72% male; 58% Black) were included in analysis. Predictor variables positively associated with disengaging from care include number of positive gonorrhea or chlamydia tests; number of recent syphilis tests (OR, 95% CI: 1.38, 1.23-1.56); ever being diagnosed with syphilis; a positive amphetamines test; and schizophrenia diagnosis (OR, 95% CI: 2.63, 1.18-6.78). Predictors negatively associated with disengagement from care are higher age (OR, 95% CI: 0.98, 0.96-0.99); number of recent gonorrhea/chlamydia tests taken (OR, 95% CI: 0.77, 0.73-0.81); 2+ emergency visits; 1+ hospital admissions; 2+ hospital admissions (OR, 95% CI: 0.2, 0.06-0.67); depression diagnosis (OR, 95% CI: 0.63, 0.45-0.87); and visiting the HIV clinic in each half of the year (OR, 95% CI: 0.2, 0.15-0.27). A logistic regression model using these predictors for the testing set results in an AUC of 0.78.

**Conclusion**: A model including demographics, STD diagnoses, mental health diagnosis, and healthcare utilization behavior variables effectively identifies PLWH at-risk for care disengagement.

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**674 PARTNER TESTING AMONG PREGNANT WOMEN OFFERED HIV TESTING IN KENYA**

Wenwen Jiang¹, Peninah Kitao², Shiza Farid³, Daniel Matemo³, Anjuli Wagner³, Cheryl C. Johnson¹, Grace John-Stewart¹, John Kinuthia³, Alison L. Drake³, David Katz³

¹University of Washington, Seattle, WA, USA, ²Kenyatta National Hospital, Nairobi, Kenya, ³World Health Organization, Geneva, Switzerland

**Background**: Near-universal HIV testing among pregnant women through maternal and child health (MCH) services provides opportunities to reach male partners for HIV testing, yet partner testing remains low. In trials, secondary distribution of HIV self-tests by pregnant women has increased partner testing, but there is limited real-world evidence on partner testing when self- and clinic testing are options.

**Methods**: At 3 Kenyan MCH clinics, we offered HIV-negative pregnant women a choice of standard clinic-based testing (CBT) or HIV self-testing at home (HBT) for HIV retesting for themselves. Women who selected HBT were offered additional self-test kits for male partner testing; while women who selected CBT could refer partners to clinic for routine testing. We assessed HIV testing uptake among male partners by 14 weeks postpartum. We used generalized linear mixed regression with Poisson distribution to (1) identify correlates of partner testing, stratified by whether women selected HBT or CBT, and (2) and partner testing via HBT (vs. CBT) among women selecting HBT.

**Results**: Among 798 women, median age was 24 years and most were cohabiting with partners (87%). By 14 weeks postpartum, 163 (60%) of 270 women who selected HBT for themselves at enrollment and 200 (38%) of 528 of women who selected CBT reported partner HIV testing (p<0.001). Of those reporting partner testing, 314 (87%) reported partner status negative, 2 positive, and 47 did not report results. Among all women (both HBT and CBT), partner testing was associated with higher maternal education and cohabiting with partners (87%). By 14 weeks postpartum, 163 (60%) of 270 women who selected HBT for themselves at enrollment and 200 (38%) of 528 of women who selected CBT reported partner HIV testing (p<0.001). Of those reporting partner testing, 314 (87%) reported partner status negative, 2 positive, and 47 did not report results. Among all women (both HBT and CBT), partner testing was associated with higher maternal education and cohabiting with partners (87%). Among 528 of women who selected CBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1). In a subset of 163 women opting for HBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1). In a subset of 163 women opting for HBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1). In a subset of 163 women opting for HBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1). In a subset of 163 women opting for HBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1). In a subset of 163 women opting for HBT, partner testing was associated with having partner >5 years older and not reporting low sexual relationship power (Table 1).
675 INNOVATIVE QUALITY APPROACH TO IMPROVE HIV RAPID TESTING IN SOUTH AFRICA

Karida Diallo1, Mireille B. Kalou1, Makhanya Mhlangosazana1, Adeboyce Adelekan1, Leigh Berrie1, Kamba N. Lee1, Kassahun Ayalew1, Joseph Honwani1, Dumisani Mhlongo2, Robert Molale2, Anil Kalan2, Amanda Mohale1, Bandile Ndizai1, Peter Manyike1

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Background: In 2014, South Africa piloted the Rapid Test Continuous Quality Improvement (RTCQI) initiative to improve HIV testing at 2,077 facilities. Preliminary data showed some improvement in pre-testing, testing, and post-testing phases, which ultimately affect the accuracy of diagnosis. To address the gaps identified during the pilot phase and improve accuracy of test results, RTCQI was implemented in 2,600 US President’s Emergency Plan for AIDS Relief (PEPFAR)-supported facilities. We report improvement achieved in a cohort of 690 randomly selected facilities.

Methods: The Stepwise Process for Improving the Quality of HIV Rapid Testing (SPI-RT) checklist, was used to assess the annual improvement in pre-defined quality standards for HIV rapid testing, including pre-testing, testing, and post-testing. Assessment results were converted to percentages, and facilities were rated (levels 0–4). Level 0 indicates improvement needed in all quality standard domains, whereas level 4 indicates readiness for national certification. Facilities’ improvement after implementing RTCQI was determined using McNemar’s test, and pre- and post-test improvements were compared using paired t-test.

Results: A cohort of 690 (26.5%) facilities, with a complete dataset from 2017 to 2020, showed improvements in pre-testing, testing, and post-testing domains. The proportion of level 4 facilities increased from 5.1% (95% CI, 3.6%–7.0%) in 2017 to 43.5% (95% CI, 40.0%–47.3%) in 2020 (p-value < 0.0001), whereas the proportion of level 1 facilities that needed improvement in some domains significantly decreased from 22.0% in 2017 to 2.0% in 2020 (Figure). The average performance score of the facilities at Level 4 for the pre-testing, testing and post-testing domains increased by 6.6% (95% CI, 5.6%–7.6%), 11.2% (95 CI, 9.6–12.7) and 5.7% (95% CI, 4.7%–6.7%) respectively by 2020. Qualitative assessment of the data showed improvement in activities implemented directly by the providers, such as enhanced stock and consumables management, increased uptake of internal quality control measures and HIV-testing registers, and proper documentation of test results in the registers.

Conclusion: We found significant improvement in quality standards in facilities offering HIV RT that implemented the RTCQI. These improvements were due to monitoring HIV-testing quality standards as part of the HIV Testing Services’ policy and allocating human resources at the provincial level to oversee the implementation of and adherence to these standards.
678 USE OF MULTI-ASSAY ALGORITHMS TO IDENTIFY RECENT HIV INFECTIONS: HPTN 071/POPART

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1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 3FH 360, Durham, NC, USA, Zambart, Lusaka, Zambia, 1Desmoult Tuberculosis Unit, Western Cape, South Africa, 2Imperial College London, London, UK, 3London School of Hygiene & Tropical Medicine, London, UK

Background: Multi-assay algorithms (MAAs) developed for estimating HIV incidence from population-level surveys have also been used to identify individuals with recent infection. Little is known about the performance of these methods for individual-level recency assessment. The most widely used MAA for incidence estimation includes the limiting antigen avidity assay

(LAg-Avidity) plus HIV viral load (CDC MAA). We compared the performance of the CDC MAA to three other MAAs for identifying persons infected <1 year as recently infected.

Methods: This study included samples from 220 seroconverters (infected <1 year) and 4,336 non-seroconverters (infected >1 year) enrolled in an HIV prevention trial in Zambia and South Africa, 28.6% of seroconverters and 73.4% of non-seroconverters were virally suppressed at sample collection (viral load <400 copies/mL). Samples were tested using two laboratory-based assays (LAG-Avidity, BioRad-Avidity) and a point-of-care assay (Assante HIV-1 Rapid Recency assay; rapid LAg). Four MAAs were evaluated that included different combinations of assays with different assay cutoffs (Table).

Results: The four MAAs classified different numbers of seroconverters (54 [24.5%] to 100 [45.5%]) and non-seroconverters (11 [0.3%] to 69 [1.6%]) as recently-infected. Sensitivity, specificity, and the false recent rate for identifying seroconverters as recent varied among the MAAs (Table). Two MAAs classified fewer seroconverters as recent than expected based on their median window periods (CDC MAA, p=0.0004; Clade C MAA, p <0.0001). The 60 seroconverters classified as recent by the CDC MAA were a subset of the 100 seroconverters classified as recent by the Clade C MAA. Seventy-two seroconverters were classified as recent by the CDC MAA and/or the Rapid LAg MAA; these MAAs use different LAg assays with the same viral load cutoff. These 72 seroconverters included 12 classified as recent by the Rapid LAg MAA only, 18 identified as recent by the CDC MAA only, and 42 classified as recent by both MAAs.

Conclusion: Substantial differences were observed in the performance of the four MAAs for identifying individuals infected <1 year as recently infected. Each MAA classified different groups of individuals as recent vs. non-recent, even when the MAAs differed only in the type of LAg assay used (lab-based assay vs. rapid). These performance issues should be considered when using these MAAs for applications designed to identify individuals with recent HIV infection.

Table: Performance of MAAs for classifying persons infected <1 year as recently infected.

<table>
<thead>
<tr>
<th>MAA</th>
<th>CDC MAA</th>
<th>Clade C MAA</th>
<th>Rapid LAg MAA</th>
<th>Alternate MAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>72.3%</td>
<td>57.3%</td>
<td>45.6%</td>
<td>68.4%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.6%</td>
<td>98.4%</td>
<td>98.7%</td>
<td>98.4%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>84.6%</td>
<td>74.6%</td>
<td>76.6%</td>
<td>54.0%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98.5%</td>
<td>97.3%</td>
<td>98.6%</td>
<td>97.9%</td>
</tr>
<tr>
<td>False recent rate (infected &gt;2 years)</td>
<td>0.2%</td>
<td>0.5%</td>
<td>0.4%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Window period (days)</td>
<td>242</td>
<td>248</td>
<td>105</td>
<td>126</td>
</tr>
<tr>
<td>Expected recent</td>
<td>86</td>
<td>149</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td>CDC MAA: LAg+1.5 + VL &lt; 1000. Clade C MAA: LAg+2.8 + BioRad A+/VL &lt;400. Rapid LAg MAA: Rapid LAg + recent = 1/VL &lt;1000. Alternate MAA: LAg+1.5 + BioRad A+/VL &lt;400.</td>
<td></td>
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</tr>
</tbody>
</table>
680 EVALUATION OF A RAPID TEST ALGORITHM TO ESTIMATE HIV INCIDENCE: HPTN 071/PopART

Ethan Klock1, Ethan A. Wilson2, Reinaldo Fernandez3, Denni Lennon4, Ayana Moore5, Barry Kosloff6, Richard Hayes7, Ronald M. Kate Grabowski8, Joseph Kagaayi9, Deborah Peter10, Peter Bock11, Susan Eshleman12, Helen Ayles13, Oliver Laeyendecker14, Anneen Van Deventer15
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Background: Lateral flow point-of-care tests (POCTs) designed to distinguish recent from long-term HIV infection surveillance data suggested that Chirundu, a Zambian border town with Zimbabwe, may be a hot spot district where CRS EpC 3-90, MOH and other stakeholders should prioritize for HIV preventive and treatment interventions.

Methods: We assessed the performance of an algorithm incorporating the HIV-1 LAg-Avidity EIA (LAg) and rapid test for HIV 1 and 2 (HIV-1+2) VITROS platform (Asante) in a community randomized trial (HPTN 071/PopART) in Chirundu, Zambia from 2017-2019. Participants were 18 years or older, with between 12 and 24-month study visits. READ: The percent of recent infections by study arm, country, sex and age group.

Results: Seventy-two percent of participants were female (n=2461). The mean age was 29.3 years old (SD=7.3). The majority of participants were from Chirundu (78%). Risk behaviors included: male sex work (8.4%), sex work (12.7%), unprotected anal sex (60.7%), and mass infant feeding (16.7%). The median duration of study visit was 24 months (IQR=12-36 months). The rapid test algorithm had a sensitivity of 80.9% (95% CI: 75.3-85.2) and specificity of 99.4% (95% CI: 99.0-99.8). The overall incidence of recent infection was 6.0% (95% CI: 5.0-7.1) estimated cross-sectionally at the 24-month study visit for all participants. The overall incidence estimate for the LAg+VL algorithm was 4% lower than observed HIV incidence. In contrast, the overall incidence estimate for the LAg+VL algorithm was 4% lower than observed incidence.

Conclusion: The Rapid+VL testing algorithm underestimated HIV incidence in a large population cohort from South Africa and Zambia. This suggests that the MDRI recommended by the manufacturer is too long or that the assay is not accurately detecting a sufficient portion of the recent infections. Additional studies are needed to determine the correct MDRI for this cross-sectional incidence algorithm.

Table: Comparison of Incidence Algorithms for HIV Incidence Estimation

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Incidence (per 100 person years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed (95% CI)</td>
</tr>
<tr>
<td>All</td>
<td>1.34 (1.17, 1.53)</td>
</tr>
<tr>
<td>Study Arm</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.14 (1.03, 1.27)</td>
</tr>
<tr>
<td>B</td>
<td>1.07 (0.92, 1.25)</td>
</tr>
<tr>
<td>C</td>
<td>1.08 (0.93, 1.25)</td>
</tr>
<tr>
<td>Country</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>1.23 (1.02, 1.46)</td>
</tr>
<tr>
<td>Zambia</td>
<td>1.43 (1.18, 1.67)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.65 (1.42, 1.89)</td>
</tr>
<tr>
<td>Male</td>
<td>0.64 (0.44, 0.93)</td>
</tr>
<tr>
<td>18-24 yrs old</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.14 (1.69, 2.67)</td>
</tr>
<tr>
<td>Male</td>
<td>0.57 (0.28, 1.02)</td>
</tr>
</tbody>
</table>

681 COMPARISON OF ASANTE AND SWIFT HIV RECENT INFECTION ASSAYS

Haley Schmidt1, Reinaldo Fernandez2, Ethan U. Patel3, Charles Morrison4, Ronald Galli1, Ethan A. Wilson2, Reinaldo Fernandez3, Joseph Kagaayi4, M. Kate Grabowski5, Aaron Tobian6, Andrew Redd4, Thomas Quinn1, Oliver Laeyendecker1
1Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 3Behavioral Epidemiology and Clinical Sciences, FHI, Durham, NC, USA, 4Rakai Health Sciences Program, Rakai, Uganda, 5National Institutes of Health, Bethesda, MD, USA

Background: Lateral flow point-of-care tests (POCTs) designed to distinguish between recent and long-term HIV-1 infections could increase the feasibility of monitoring population-level HIV transmission in real-time; however, their performance has not been well characterized. We evaluated the performance of the Swift Recent Infection Assay (Swift) and the Asante HIV-1 Rapid Recency Assay (Asante) in a panel of specimens with known duration of infection, and compared their results to the LAg-Avidity EIA (LAg).

Methods: We assessed the performance of 254 samples from 141 HIV-positive subjects. This included 26 subjects, half infected with subtype C and half with subtype D. 168 samples from <6 months; 48 samples from 6 to 12 months; 43 samples >1 year after seroconversion. An additional 115 samples were from long-term infected subjects (3 to 17 years) from Rakai, Uganda. Assays were conducted per manufacturer protocol. Swift bands were read visually and with the manufacturer’s digital reader, while Asante bands were read visually. The lack of a long-term band and viral load <1000 copies/mL were necessary for the classification of recent infection. Cohen’s kappa statistics (k) were used to assess assay agreement.

Results: There was differential classification for recent infection by subtype for both POCTs, which was not seen for LAg (n=139) (Figure). Samples from individuals infected with subtype C were correctly classified as recent less frequently than individuals infected with subtype D in the first 6 months (27% vs 85%) or at 6 to 12 months post seroconversion (0% vs 40%). Among long-term infected individuals (>1 year) there was little difference seen between subtypes. For the Rakai samples (n=115), ~4% were misclassified by each POCT method. Overall for the Swift (n=254), results generated by visual assessment and the digital reader, had high agreement (96%; k=0.85). Agreement between visual Swift and LAg was 83% (k=0.50), while agreement between digital reader Swift and LAg was 81% (k=0.45). Agreement between visual Swift and Asante was 92% (k=0.73). The agreement between Asante and LAg was 83% (k=0.52). Of note, the lowest agreement between POCTs and LAg was seen in long-term Rakai samples (78%, k=0.17).

Conclusion: Differential classification of recent infection occurred by subtype for both POCTs. Agreement between visual and digital reader classification for the Swift was high. The classifications of POCTs to LAg were similar, though lowest seen in long-term samples from Rakai.
682 IMPACT OF EARLY VIRAL SUPPRESSION ON HIV INCIDENCE CASES: HPTN 071 (PopART)

Wendy Grant-McAuley, Ethan Klock, Oliver Layendeckers, Estelle M. Piwowar-Manning, Ethan A. Wilson, William Clarke, Autumn Beaud, Ayana Moore, Helen Ayles, Peter Bock, Deborah Donnelli, Sarah Fidler, Richard Hayes, Susan Ehleman, for the HPTN 071 (PopART) Study Team

The Johns Hopkins University School of Medicine, Baltimore, MD, USA; Fred Hutchinson Cancer Research Center, Seattle, WA, USA; FH 360, Durham, NC, USA; Zambart, Lusaka, Zambia; Desmond Tutu TB Centre, Western Cape, South Africa; Imperial College London, London, UK; London School of Hygiene & Tropical Medicine, London, UK

Background: Serologic assays used for cross-sectional HIV incidence estimation measure general characteristics of the HIV antibody response. Viral suppression is known to impact the performance of these assays in persons with established infection. Frequent HIV testing and universal antiretroviral treatment (ART) helps many individuals achieve viral suppression in the first year of infection. Little is known about the impact of early viral suppression on the performance of these assays. We evaluated the performance of incidence assays using seroconverter samples from a community-randomized trial that evaluated the impact of universal testing and treatment on HIV incidence (HPTN 071 [PopART]).

Methods: This study included samples from 219 study participants who were infected <1 year (seroconverters); 62 (28.3%) of the seroconverters were virally suppressed (viral load ≤400 copies/mL). Samples were tested using two laboratory-based assays (LAg-Avidity, BioRad-Avidity) and a point-of-care assay (Assanté HIV-1 Rapid Recency assay). A qualitative multi-drug assay was used to identify seroconverters on ART.

Results: Antiretroviral (ARV) drugs were detected in 49 (79%) of the 62 virally suppressed seroconverters indicating that they were on ART. Those suppressed on ART were more likely to have higher BioRad-Avidity values than viremic seroconverters (p = 0.021), consistent with the expected longer duration of HIV infection in those who were suppressed on ART by their first HIV-positive visit. In contrast, seroconverters who were suppressed on ART were more likely to have LAg-Avidity values <1.5 and less likely to have the long-term infected band on the rapid LAg assay than those who were viremic (p = 0.0096, p = 0.00047, respectively). Seroconverters suppressed on ART were also more likely to have LAg-Avidity values <1.5 than those who were virally suppressed in the absence of ARV drugs (p = 0.014).

Conclusion: Early ART was not associated with lower BioRad-Avidity values. In contrast, individuals who started ART and were suppressed within the first year of infection were more likely to have lower reactivity as measured by the LAg-Avidity and Rapid LAg assays than those who were viremic. This may reflect down-regulation of HIV antibodies in those on ART. Lower LAg-Avidity values were also obtained more frequently when viral suppression was due to ARV drug use. This may reflect differences in antibody expression in those with natural vs. ARV-induced viral suppression.

683 MISSED HIV & HCV SCREENING IN EMERGENCY-DEPARTMENT PATIENTS WITH OPIOD-USE DISORDER

Michael S. Lyons, Marek C. Chawarski, Richard Rothman, Lauren Whiteside, Ethan Cowan, Lynne D. Richardson, Kathryn Hawk, Judith I. Tsui, Robert P. Schwartz, Patrick O’Connor, Gail D’Onofrio, David A. Fiellin, Jennifer E. Edelman

University of Cincinnati, Cincinnati, OH, USA; Yale University, New Haven, CT, USA; Johns Hopkins University School of Medicine, Baltimore, MD, USA; University of Washington, Seattle, WA, USA; Vah School of Medicine at Mount Sinai, New York, NY, USA; IBM Research—Zurich, Zurich, Switzerland; University of Washington School of Medicine, Seattle, WA, USA; Friends Research Institute, Baltimore, MD, USA; Yale School of Medicine, New Haven, CT, USA

Background: Individuals with untreated opioid use disorder (OUD) are at substantial risk for HIV and hepatitis C virus (HCV). Emergency departments (EDs) provide a promising opportunity to screen high-risk populations and have made progress in screening program implementation. We evaluated HIV and HCV screening among ED patients with untreated OUD.

Methods: This cross-sectional analysis used data from a multi-site, hybrid type III effectiveness-implementation study to promote buprenorphine initiation in four large, urban, academic EDs. Structured screening programs, separate from but concurrent with the primary study, were in place for HIV at all sites and HCV at three sites. Consenting participants, enrolled between February 2017 and January 2019, were adult, English-speaking ED patients who met Diagnostic and Statistical Manual (DSM)-5 criteria for OUD and were not receiving addiction treatment. Study assessments included self-reported sociodemographics, presence of medical provider for usual care, self-reported HIV and HCV status, and HIV/HCV related risk behaviors, as well as chart review to determine receipt of HIV or HCV screening during the ED encounter at which study enrollment occurred. Individuals reporting both HIV and HCV infection were excluded from analysis. Descriptive statistics were used to determine the proportion tested overall, by injection drug use (IDU) in the past month, and by ED site.

Results: Among 394 enrolled participants, 38% reported having a medical provider for usual care. There were 375 without reported HIV positive status, of whom 59 (16%) received an ED HIV test. Of 218 participants without known HIV who reported IDU, 9 (9%) were tested for HIV. There were 231 without reported HCV positive status, of whom 59 (16%) received an ED HCV test. Of 98 participants without known HCV who reported IDU, 9 (9%) were tested for HCV. The proportion tested across study sites ranged from 3% to 25% for HIV and 4% to 32% for HCV.

Conclusion: ED HIV and HCV screening remains insufficient among patients with untreated OUD, including those who inject drugs, even in ED settings with formal screening programs. Targeted HIV/HCV screening in EDs should be implemented as an important adjunct strategy until the ideal of universal screening can be more fully achieved, particularly given low rates of routine medical care in this population.

684 SUSTAINED 97% HIV TESTING RATE IN THE EMERGENCY DEPARTMENT: THE NEW GOLD STANDARD

Linda Cheyenne Vaccari, Sarah Parry, Deborah Kirkham, Steven Pike, Leslie Perry, Andrew Widdowsen, Sarah Horne, Ian Cormack

Croydon University Hospital, London, UK

Background: UK 2020 HIV guidelines recommend opt-out HIV testing in Emergency Departments (ED) in areas of high prevalence (>2/1000). Our area has a very high HIV prevalence (>5/1000) with a 46% late diagnosis rate. We implemented opt-out testing in our ED in May 2020, sustaining testing rates of 97%.

Methods: All patients aged ≥16 undergoing venesection in ED have an HIV test automatically added. A separate blood sample is tested using Roche 4th generation HIV 1/2 antigen-antibody combination test. Posters and leaflets are prominently displayed in ED, signposting how to opt-out. IT blocks duplicate testing and those opted-out within the past 6 months. The HIV team receives an alert. Consent is prominently displayed in ED, signposting how to opt-out. IT blocks duplicate testing and those opted-out within the past 6 months. The HIV team receives an alert. Consent is prominently displayed in ED, signposting how to opt-out. IT blocks duplicate testing and those opted-out within the past 6 months. The HIV team receives an alert.

Results: Among 24,621/25,336 (97%) eligible patients were tested. This data excludes 214 patients who were already engaged in care. 14 had defaulted care; nine have now re-engaged. 15 patients were confirmed new HIV diagnoses; 13 are
now engaged in care and receiving antiretrovirals and two have declined care. 8/14 (57%) new patients and 5/9 (56%) defaulters had a CD4 count <200. 9/13 (69%) new patients had missed diagnostic opportunities. 42/244 (17%) patients with reactive tests were verified as false positives. 12 patients are awaiting repeat testing. Seven regular partners of newly diagnosed patients were verified HIV negative and managed with post- or pre-exposure prophylaxis, condoms and/or treatment as prevention. In the same period, ED diagnosed 15 patients compared to 12 non-ED (eight sexual health, one antenatal, two haematology, one medical). Our tested ED HIV prevalence is 7.72/1000 compared to a local recorded prevalence of 5.84/1000 (p<0.0002).

**Conclusion:** Collaborative working between ED, pathology, IT and HIV can sustain 97% testing rates using opt-out testing. The prevalence of HIV in ED attendees is statistically significantly higher than the local prevalence underlying the importance of HIV testing in ED. Wider benefits include earlier HIV diagnosis, reduced morbidity, mortality, investigations and healthcare costs, reduced length of stay, and reduced onward transmission.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Birth Country</th>
<th>Reason for ED attendance</th>
<th>CD4 [%]</th>
<th>HIV Viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>28</td>
<td>Ghana</td>
<td>Gastroenterology</td>
<td>732 (46)</td>
<td>&lt;50</td>
</tr>
<tr>
<td>M</td>
<td>34</td>
<td>UK</td>
<td>Psychiatric</td>
<td>537 (76)</td>
<td>255,000</td>
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<tr>
<td>M</td>
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<td>Portugal</td>
<td>Gastroenterology</td>
<td>181 (16)</td>
<td>413,000</td>
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<tr>
<td>M</td>
<td>28</td>
<td>UK</td>
<td>Trauma</td>
<td>1180 (10)</td>
<td>368</td>
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<tr>
<td>F</td>
<td>35</td>
<td>Cameroon</td>
<td>Neurology</td>
<td>374 (17)</td>
<td>12,200</td>
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<td>M</td>
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<td>M</td>
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<td>Nigeria</td>
<td>Neurology</td>
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<tr>
<td>M</td>
<td>43</td>
<td>Portugal</td>
<td>Ophthalmology/Dermatology</td>
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<td>F</td>
<td>22</td>
<td>Ghana</td>
<td>Gynaecology</td>
<td>742 (64)</td>
<td>1,930</td>
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<tr>
<td>M</td>
<td>31</td>
<td>Brazil</td>
<td>Neurology</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>M</td>
<td>48</td>
<td>Brazil</td>
<td>Infection</td>
<td>372 (17)</td>
<td>300,000</td>
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<tr>
<td>M</td>
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<td>Germany</td>
<td>Infection/Dermatology</td>
<td>84 (7)</td>
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</tr>
<tr>
<td>F</td>
<td>54</td>
<td>Ghana</td>
<td>Multisystem</td>
<td>167 (12)</td>
<td>337,000</td>
</tr>
<tr>
<td>M</td>
<td>53</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unreported</td>
<td>&lt;50</td>
</tr>
<tr>
<td>F</td>
<td>36</td>
<td>Cameroon</td>
<td>Gynaecology</td>
<td>188 (15)</td>
<td>23,200</td>
</tr>
</tbody>
</table>

**Table 1:** Demographics and baseline information for newly diagnosed patients

**685 HIV/STI TESTING AND PrEP ELIGIBILITY AMONG WOMEN INCARCERATED IN AN URBAN COUNTY JAIL**

Jui Desai¹, Ank Nijhawan², Douglas Krakower³, Barry-Lewis Harris⁴, Dena Taherzadeh²

¹Parkland Health and Hospital Systems, Dallas, TX, USA, ²University of Texas Southwestern, Dallas, TX, USA, ³Beth Israel Deaconess Medical Center, Boston, MA, USA

**Background:** Women in the criminal justice (CJ) system experience higher rates of HIV infection compared to both men in the CJ system and non-CJ involved women, due to high-risk factors and are eligible for pre-exposure prophylaxis (PrEP), though limited data exist on the implementation of PrEP in this population.

**Methods:** The results of all Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) urine and HIV/Syphilis testing in women in the Dallas County Jail (DCJ) were compiled from January to October 2020. Electronic medical records (EMR) from the DCJ for a month-long period (March 2020) were manually reviewed and data collected regarding age, substance use, homelessness, patient request for STI testing, and time between GC/CT and HIV/syphilis results.

**Results:** From January to October 2020, 4398 females were tested for CT and 4389 for GC, and among this group, 479 (11%) were also tested for HIV and 562 (13%) for syphilis. Of women tested, median age was 42, and 462/4398 (11%) were positive for CT, 323/4389 (7%) positive for GC, 10/479 (2%) women had positive HIV tests, of whom 6 (1.3%) were new diagnoses and 75 (1.7%) had a reactive rapid plasma regain test. In March, 541 women were tested for CT and 438 women for GC, 90 of whom tested positive for either CT or GC. Of these 90, the vast majority, 70 (78%) did not receive testing for HIV or syphilis, including women with the following risk factors: 4 (6%) were pregnant, 10 (14%) were homeless, 19 (27%) had requested STI/HIV testing at jail intake, 11 (16%) reported heroin use and 10 (14%) reported methamphetamine use. Individuals tested for all four infections (CT/GC/HIV/syphilis) were incarcerated for a longer period of time compared to those not tested for HIV/syphilis (83 ± 30 days), with median time to HIV/syphilis testing after GC/CT result at 11 days.

**Conclusion:** Women incarcerated at a county jail had high rates of STIs and multiple other HIV risk factors, though only 1 in 5 with acute STIs (11%) overall were tested for HIV or syphilis. Efforts to improve HIV prevention in this high-risk, vulnerable population should include expansion of HIV testing, through paired STI and HIV testing offered early during incarceration, and utilization of automated EMR tools to highlight women who are PrEP candidates, like those testing positive for bacterial STIs or those with active substance use. Identification of those with elevated HIV risk, followed by counseling and linkage to PrEP care, could have a major impact on HIV prevention for incarcerated women.

**Implementation and Results of STI Testing From January 2020-October 2020 in Female Inmates in Dallas County Jail**

**868 ARE WE REACHING the AT-RISK PATIENT POPULATIONS FOR HIV AND OTHER STIs?**

Christoph Boesecke¹, Torben Kimhofer², Christopher Tocha³, Oliver Schubert³, Rainer Rybak², Klaus Kuhlen³, Christoph Klages³, Jürgen K. Rockstroh³

¹Bonn University Hospital, Bonn, Germany, ²Murdoch University, Murdoch, Australia, ³AIDS-Hilfe Cologne, Cologne, Germany

**Background:** Published clinical and epidemiological data on individuals persons undergoing anonymous testing for sexually transmitted diseases (STI) in Germany are sparse. Here we report annual results of STI screenings and survey data from a large community based STI testing Checkpoint in Cologne, Germany.

**Methods:** From January 2017 to December 2019, data on STI screening, clinical, demographic, sexual information was anonymously recorded for individuals attending the Checkpoint in Cologne. Visitors were screened for HIV, syphilis, chlamydia, gonorrhoea and hepatitis C using point of care testing kits. Positive tests were validated.

**Results:** Overall, screening was performed on 11,456 visitors aged 16 to 85 years. Three main reasons were identified: recent HIV risk situation (46%), routine testing (29%), beginning of a new relationship (24%). The largest visitor group constituted men who have sex with men (MSM, 44%), followed by men who have sex with women (MSW, 29%), women having sex with men (WMS, 22%). MSM engaged on average with a higher number of sex-partners than MSW and WSM with 36% having 2-5 sex-partners and 10% having ≥ 26 per year. The annual number of visitors on PrEP (96% MSM) increased steadily, with a total of 29 visitors in 2017, 54 in 2018 and 123 in 2019 (p<0.001). The MSM group had the highest disease frequency (chlamydia: 140, gonorrhea: 123, syphilis: 88, HIV: 56, HCV: 2). STI frequency in PrEP users was highest for chlamydia/gonorrhoea (7%, 9% and 12% for 2017, 2018 and 2019, respectively). The largest visitor group constituted men who have sex with men (MSM, 44%), followed by men who have sex with women (MSW, 29%), women having sex with men (WMS, 22%). MSM engaged on average with a higher number of sex-partners than MSW and WSM with 36% having 2-5 sex-partners and 10% having ≥ 26 per year. The annual number of visitors on PrEP (96% MSM) increased steadily, with a total of 29 visitors in 2017, 54 in 2018 and 123 in 2019 (p<0.001). The MSM group had the highest disease frequency (chlamydia: 140, gonorrhea: 123, syphilis: 88, HIV: 56, HCV: 2). STI frequency in PrEP users was highest for chlamydia/gonorrhoea (7%, 9% and 12% for 2017, 2018 and 2019, respectively). One PrEP user tested positive for HIV. In all other groups, chlamydia was the most prevalent infection while both HCV cases occurred among MSM. Despite increased PrEP prevalence in Germany since 2017 (covered by health insurance from 09/2019 onwards) no decline in HIV infection rates was observed with 20, 29 and 24 cases in 2017, 2018 and 2019, respectively (p=0.46). 56 new HIV infections were seen in MSM, 4 in MSW, 7 in WSM. 58% of newly diagnosed individuals indicated that a significant risk situation had occurred in the recent past, followed by 33% indicating routine testing.

**Conclusion:** Checkpoint was able to detect relevant STIs in 5% of all visitors thereby underlying the importance of community-based testing sites. Despite increased HIV awareness and PrEP roll-out MSM remain at highest risk for contracting HIV (and HCV) highlighting the continuous need for educational activities as well as low-threshold and cost-free STI screening capacities.
DECREASED HIV TESTING BEFORE HIV DIAGNOSIS AMONG BLACKS/AFRICAN AMERICANS: US 2013-18
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1Centers for Disease Control and Prevention, Atlanta, GA, USA. *F International, Atlanta, GA, USA

Background: In 2018, Blacks/African Americans (Blacks) accounted for 13% of the US population and 42% of new HIV diagnoses. Ensuring high levels of HIV testing is essential in reducing disparities in HIV diagnoses and decreasing the rate of HIV infection in Blacks. Our analysis elucidates which subgroups may benefit from increased testing, leading to linkage to care and improvement in HIV care outcomes.

Methods: We used NHSS data to assess trends in HIV testing patterns among Blacks aged ≥13 years with HIV infection diagnosed from 2013–2018 by sex, age and transmission category. HIV testing history data were collected by 21 sites participating in HIV incidence surveillance. The estimated annual percent change (EAPC) and 95% CIs were used to assess trends from 2013–2018.

Results: The number of Blacks with diagnosed HIV infection was 11,742 in 2013 and 10,881 in 2018 and the percentage with any testing history was 66% in 2013 and 61% in 2018. Among Blacks with testing history, the percentage who ever had a previous negative HIV test decreased significantly from 66% in 2013 to 58% in 2018 [EAPC = -2.3,95%CI(-3.2,-1.8)]. Significant decreases occurred for males and females. By age, significant decreases occurred for those aged 13-24, 25-34 and 35-44 years. Among Blacks with infection attributed to male to male sexual contact, the percentage who had a previous negative HIV test decreased significantly from 70% to 62% [EAPC = -2.1,95%CI(-3.1,-1.4)]. Among those with infection attributed to heterosexual contact, the percentage who had a previous negative HIV test decreased significantly from 54% to 44% [EAPC = -3.8,95%CI(-6.1,-1.4)] for males and from 52% to 50% [EAPC = -3.7,95%CI(-5.2,-2.2)] for females. Among Blacks with a known negative HIV test date before HIV diagnosis, the trend in the percentage of those with a negative test ≥12 months before diagnosis remained stable overall (mean 49% per year) and was also stable for all sex, age and transmission categories.

Conclusion: There is decreased HIV testing among Blacks overall, for males and females and those with infection attributed to male to male sexual contact and heterosexual contact. The percentage with a negative test ≥12 months before diagnosis remained stable for all groups. Annual HIV testing should be promoted among Blacks at higher risk of infection to increase early detection of HIV infection, and also to increase linkage as a means for improving HIV care outcomes and reducing risk for HIV transmission.

THE PLASMA SEPARATION CARD (PSC): A SUITABLE ALTERNATIVE TO PLASMA HIV-1 VIRAL LOAD
Katrina Sleeman1, Lucia Hans1, Guoping Zhang1, Stephen Jadczyk1, Lynette Makuwazi2, Kenielwe Peloakgosi-Shikwambani1, Mackenzie Hurstlon Cox1, Heather Alexander1, Sergio Carmona2, Clement Zeh1
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2National Health Laboratory Service, Johannesburg, South Africa

Background: Cold chain and centrifugation requirements of plasma have limited the access of remote and vulnerable populations to HIV viral load (VL) testing. Roche Molecular Diagnostics developed a sample collection device, the plasma separation card (PSC) where plasma is obtained from the addition of a plasma separation card (PSC) to blood. We aimed to assess the performance of PSC as an alternative matrix for HIV-1 VL across multiple Roche testing platforms for WHO prequalification and for use in President’s Emergency Plan for AIDS Relief (PEPFAR) countries.

Methods: Performance of PSC dried plasma spots were compared to centrifugal plasma samples using HIV negative whole blood spiked with cultured virus or using the third HIV-1 International WHO Standard and remnant clinical samples submitted for HIV VL testing. Prepared dried plasma spots were tested within 56 days using the COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) HIV-1 Test v2, and the cobas® HIV-1 Test on the cobas® 4800 (c4800) and 6800/8800 (c6800/8800) platforms. The limit of detection (LOD) was calculated using PROBIT analysis. The precision of measurement, cross-contamination, and linearity for subtypes A, B, C, D and CRF02-AG were also determined. Bland-Altman and correlation analysis were applied to evaluate clinical performance of the PSC as an alternative matrix for HIV-1 VL across multiple Roche testing platforms for WHO prequalification and for use in President’s Emergency Plan for AIDS Relief (PEPFAR) countries.

Results: The LOD for PSC samples was calculated to be 579.3, 745.2, and 489.4 copies/mL on the CAP/CTM, c4800 and c6800/8800 respectively. No cross-contamination was detected on any platform among the 40 samples tested, alternating between 20 high positive and 20 negative samples. Standard deviation between runs for all three platforms was within 0.14 log10 copies/mL. Linearity assessment of HIV-1 subtypes A, B, C, D and CRF02-AG showed R2 values greater than 0.98 for all three platforms. Invalid rates using the PSC were below 5%. When compared to centrifuged plasma, the PSC had a demonstrated sensitivity of 96% and 95% and a specificity of 94% and 97% on the c4800 and c6800/8800 respectively. The average bias between PSC and plasma was less than 0.2 log10 copies/mL for the c4800 and c6800/8800 respectively.

Conclusion: These findings reveal the PSC as a suitable alternative to plasma HIV-1 VL for monitoring patients on antiretroviral treatments in remote settings and in low- and middle-income countries. This PSC evaluation contributed to WHO prequalification and PEPFAR approval.

TIMELY HIV CASE AND SEQUENCE REPORTING FOR CLUSTER DETECTION: UNITED STATES
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1Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Ending the HIV Epidemic (EHE) initiative, requires timely HIV case and sequence reporting. Expediting reporting of case reports, needed to detect clusters and causative factors, is essential in reducing disparities in HIV diagnoses and decreasing the rate of HIV infection. Sequence results, which has implications for detecting molecular clusters, is driven by reporting rather than physician ordering of drug resistance testing, suggesting potential for improvement with changes in laboratory reporting and informatics practices. Additional EHE funding can be used to improve processes to expedite reporting and entry of case, laboratory, and key data into surveillance systems for real-time decision-making.

Methods: Using data reported to the U.S. National HIV Surveillance System (NHSS), we assessed the timeliness of HIV case and sequence reporting to health departments overall and by geographic characteristics. Timely case reporting was defined as entry of cases ≤30 days of diagnosis, and entry of sequence reports within 45 days of diagnosis. However, only 6% had sequence data entered into the surveillance system within 45 days (median: 15 days; IQR: 0–30) was shorter than time from specimen collection to receipt and by the health department (median: 48 days; IQR: 27–70).

Table. Timely HIV case and sequence reporting of HIV diagnoses in persons >13 years in the United States in 2018

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*Q: Ending the HIV epidemic; a list of 10 jurisdictions can be accessed here: https://www.cdc.gov/hiv/basics/overview.html#ending-the-hiv-epidemic.
CORONACHEK SARS-CoV-2 POINT-OF-CARE ANTIBODY TEST PERFORMANCE IN UGANDA AND BALTIMORE

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Background: The performance of serological antibody tests to SARS-CoV-2 infection varies widely and is little known about their performance in Africa. We assessed the performance of CoronaCHEK Lateral Flow Point of Care Tests on samples from Rakai, Uganda and Baltimore, Maryland, USA.

Methods: Samples from subjects known to be SARS-CoV-2 PCR+ (Uganda: 50 samples from 50 individuals, and Baltimore: 266 samples from 38 individuals) and samples from pre-pandemic individuals collected prior to 2019 (Uganda: 1077 samples, Baltimore: 580 samples) were analyzed with the CoronaCHEK assay per manufacturers protocol. Sensitivity by duration of infection and specificity among pre-pandemic samples were assessed for the IgM and IgG bands separately and for any reactivity. Poisson regression models were used to calculate prevalence ratios (PR) for factors associated with a false-positive test among pre-pandemic samples.

Results: In Baltimore samples, sensitivity for any reactivity increased with duration of infection with 39% (95% CI 30, 49) during 0-7 days since first positive PCR, 86% (95% CI 79, 92) for 8-14 days, and 100% (95% CI 89,100) after 15 days (See Figure). In Uganda, sensitivity was 100% (95% CI 61,100) during 0-7 days, 75% (95% CI 53, 89) for 8-14 days, and 87% (95% CI 55, 97) after 14 days since first positive PCR. Specificity results among pre-pandemic samples from Uganda was 96.5% (95% CI 97, 95.2), significantly lower than the 99.3% (95% CI 98.2, 99.8) observed in samples from Baltimore (p<0.01). In Ugandan samples, individuals with a false positive result were more likely to have had a fever more than a month prior to sample acquisition (PR 2.9, 95% CI 1.1, 7.0).

Conclusion: Sensitivity of the CoronaCHEK appeared to be significantly lower in Ugandan samples from individuals within their first week of infection compared to their Baltimorean counterparts. By the second week of infection the sensitivity appeared the same between geographic areas. The specificity was significantly lower in Ugandan samples than those from Baltimore. False positive results from pre-pandemic Uganda appear to be correlated with the convalescent disease state, potentially indicative of a highly cross-reactive immune response in these individuals from East Africa.

COST-EFFECTIVENESS OF SARS-CoV-2 RAPID ANTIGEN TESTING IN LOW-RESOURCE SETTINGS

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Background: The World Health Organization (WHO) has called for increased testing to help arrest the transmission of coronavirus disease 19 (COVID-19). Molecular testing (PCR) is the recommended method for the diagnosis of COVID-19. In low-resource settings (LRS) however, the availability and public health impact of these tests is constrained by availability of testing capacity, shortages of reagents/supplies, lack of skilled personnel, long turnaround times (TAT), and costs. Despite lower sensitivity, antigen detection rapid diagnostic tests (AgRDTs) could provide improved access at lower costs and quicker TAT. We evaluated the optimal use of AgRDTs to increase testing access within TAT and reduce the cost and the number of cases missed in LRS.

Methods: We modeled estimated COVID-19 testing demand coverage based on current PCR capacity in three different epidemic phases across five African countries (Strategy 1). We then modelled five additional testing strategies that utilized a combination of PCR and AgRDT: 2) replacing current PCR coverage with AgRDT; 3) saturating testing demand with AgRDT only; 4) saturating testing demand first with PCR then the reminder with AgRDT; 5) saturating testing demand with AgRDT and reflex testing with PCR for patients at risk of severe disease; 6) constrained by budget of Scenario 1, using a mix of PCR and AgRDT. We estimated the total number of correct test results expected within a 48hr TAT, corresponding costs (assuming $12/PCR and $6/AgRDT), and the incremental cost-effectiveness ratios for each strategy and epidemic phase by country.

Results: Across all countries and phase of epidemic, there was insufficient PCR capacity to meet the calculated required testing demand within a 48hr TAT (ranging from 0-20%) (Figure). In no instance was the base case strategy that was limited to current PCR capacity considered cost-effective (CE). Strategy 3, in which testing demand was saturated with AgRDT, was considered robustly CE in every epidemic phase (54-77 per additional person with a correct test result within 48hr TAT), and would require both a large increase in budget and wide AgRDT availability. Additional strategies on the CE frontier were country and epidemic-phase specific.

Conclusion: Inclusion of AgRDT in testing strategies is CE and critical in increasing timely testing access in countries with low PCR capacity. Given the importance of timely results for epidemic control, future work should quantify the epidemic impact of saturating testing demand in LRS.

CANT’T WORK FROM HOME: POOLED NUCLEIC ACID TESTING OF LABORATORY WORKERS DURING COVID

Stephen A. Rawlings1, Brianna Scott1, Laura Layman1, Pramod Narain1, Roy Heltsley2, Caroline Ignacio3, Magali Porrachia4, Linda S. Atayde5, Antoine Chaillon1, Davey M. Smith1

1University of California San Diego, La Jolla, CA, USA, 2Flanders Institute of Biotechnology, Flanders, Belgium, 3Flanders Institute of Biotechnology, Flanders, Belgium, 4University of California San Diego, La Jolla, CA, USA

Background: Closing labs to decrease spread of COVID-19 has impacted research progress. Serial testing could supplement other measures to help provide a safe lab environment.

Methods: Lab employees who came to work at an academic laboratory at the University of California San Diego (UCSD) were invited and consented to undergo nucleic acid testing (NAT) vqRT-PCR to detect SARS-CoV-2.
RNA (FluxErgy). Results were available within one hour. Positive pools were deconvoluted and tested individually. Cost evaluation of the pooling approach was compared to individual NAT and to institutional guidelines for lab occupancy.

**Results:** From Apr 9 to Oct 26, 2020 (28 weeks), 1,199 nasal swab samples collected from lab workers were batched in 194 pools of median size 7 [95%: 3-11]. A median of 41 tests per week [95%: 22-67] were performed in a total of 77 participants (Fig 1). 19 core staff were tested a median 54 times [95%:13-95]. Of the 194 pools, 7 (3.6%, n=47 samples) were considered positive and required repeat testing of all participant samples in the pool as confirmation. One true positive was identified before work started. That participant was referred to their primary care provider. This early detection prevented a 2-week quarantine of 7 employees. Given ~$65/hour salary per lab worker, this saved 420 hours of work and ~$26,600 in wages. Current USCD guidelines recommend decreasing staffing levels to 25% of pre-COVID-19 occupancy. Regular NAT allowed 100% staffing. Screening of lab technicians with the pooled NAT strategy over 6 months cost $25,740 but permitted 2,430 person-hours of additional work ($132,210 in wages), as compared to the recommended 75% reduction without testing. A similar approach with individual NAT would cost $124,020 (thus $98,280 saved by pooling).

**Conclusion:** Regular pooled NAT for SARS-CoV-2 among lab personnel offers a cost-efficient way to maintain a safe lab environment without a reduction in staffing. This approach could be applied in other settings to help ensure safe


e work environments.

**Figure 1:** Number of SARS-CoV-2 tests performed per week in the six months from April 9 to October 26, 2020 in an academic lab. Swabs were pooled from participants working in the lab. Grey bars are negative tests. Blue bars represent pools that were flagged as positive but later determined to be false positive tests. One pool (red bar, *) was positive and deconvoluted to identify a single true positive.

693 NEUTRALIZING ANTIBODY DECAY IN PATIENTS WITH MILD OR ASYMPTOMATIC COVID-19 INFECTION


1University of Siena, Department of Medical Biotechnologies, Siena, Italy, 2Belluno Hospital, Belluno, Italy, 3University of Padova, Department of Medical Biotechnologies, Padova, Italy

**Background:** Development of neutralizing antibody (Nab) is crucial for protection from SARS-CoV-2 reinfection. The aim of the study was to analyze Nab titers (NabT) and kinetics over time in a cohort of 85 unselected not hospitalized Italian subjects (pts) with COVID-19 infection, with mild or no symptoms, tested after symptoms onset or for surveillance of healthcare workers.

**Methods:** Two-fold serial dilutions of heat-inactivated sera were incubated with 100 TCID50 of SARS-CoV-2 virus (lineage B) at 37°C for 1 h in 96-well plates. Then, pre-seeded 10,000 Vero E6 cell lines per well (ATCC CRL-1586) were treated with serum-virus mixtures and incubated at 37°C. After 72h, cell viability was determined through the commercial kit Cell-titer Glo 2.0 (Promega). The NaBT was defined as the reciprocal value of the sample dilution that showed a 50% protection of virus cytopathic effect (ID50). NabT≥5 ID50 were defined as SARS-CoV-2 positive and neutralizing. Chi squared, Wilcoxon, Fisher’s exact test and Spearman’s correlation coefficient were used.

**Results:** Female were 57 (67.1%) and median age was 48 years. Pts were classified as early tested (ET, <60 days, n=40) and late tested (LT, >60 days, n=45). Overall, 30 (35.3%) pts had low (<10 ID50) NaBT, 33 (38.8%) had intermediate NaBT (ID50 11 to 50), and 22 (25.9%) had high NaBT (ID50 >51, of them >100). The frequency of each NaBT class was comparable in ET and LT.

**Conclusion:** Regular pooled NAT for SARS-CoV-2 among lab personnel offers a cost-efficient way to maintain a safe lab environment without a reduction in staffing. This approach could be applied in other settings to help ensure safe


e work environments.

**Figure 1:** Number of SARS-CoV-2 tests performed per week in the six months from April 9 to October 26, 2020 in an academic lab. Swabs were pooled from participants working in the lab. Grey bars are negative tests. Blue bars represent pools that were flagged as positive but later determined to be false positive tests. One pool (red bar, *) was positive and deconvoluted to identify a single true positive.

694 SARS-CoV-2 TESTING IN FLORIDA, ILLINOIS, AND MARYLAND: ACCESS AND BARRIERS


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**Background:** Rapid detection and isolation of SARS-CoV-2 infections is critical to mitigate the pandemic; however, testing access across the US has been uneven and data on barriers to testing are limited.

**Methods:** We conducted serial cross-sectional assessments of experiences around SARS-CoV-2 PCR testing in Florida, Illinois, and Maryland. We sampled ~1000/state using an online survey from Jul 15–31 and Sep 16–Oct 15, 2020, with additional waves planned at 6–8 week intervals. At the time of surveys, there were no systematic differences in testing availability (public, private and free testing options) across these states. Participants were recruited using on online panel; demographic targets were provided to match age, sex, race/ethnicity and income distributions of each state. Participants were ≥18 years, provided consent, and resided in the study state. The survey covered demographics, symptoms, and PCR testing in the prior 2 weeks.

**Results:** Of 3,058 persons surveyed most recently (Sep 16–Oct 15), 316 (10%) reported wanting/need a test in the prior two weeks. Median age of participants wanting/need a test was 36 years and 46% were female; 47% self-identified as White and 57% reported working outside home. Of 316 who wanted/needed a test in the prior 2 weeks, 53% were able to get tested, of whom, 94% received results, with no significant differences by state (Figure); this was not substantially different from the proportion able to get tested in July (51%). Among those wanting/need a test, getting tested was significantly less common among men (aOR: 0.46) and those reporting black race (aOR: 0.53) and more common in those reporting recent travel (aOR: 3.33; all p<0.05). The primary reasons for testing were desire to know status (35%) and symptoms (28%). Among those tested, 53% had to wait ≥8 days to get a result from the time they wanted/need a test. Of those tested, 71% reported quarantining while awaiting results. An additional 146 who wanted/needed a test did not get tested; the main reasons for not testing in this group were not knowing where...
SIGNIFICANT IMPACT OF COVID-19 ON THE FIRST PILLARS OF THE HIV CARE CONTINUUM

Kathryn S. Hensley1, Carlijn Jordans1, Jan E. Van Beek1, Marion E. Vrieze1, Jeroen J. Van Kampen1, Charles A. Boucher1, Femke P. Mollena1, Elizabeth H. Gicof1, Rachida El Moussaou1, Gonneke Hamerand1, Renee N. Finkenflug1, Bart J. Rijnders1, Annelies Verbon1, Casper Rokx1

1Erasmus University Medical Center, Rotterdam, Netherlands, 2Haaglanden Medical Centre, The Hague, Netherlands, 3Rijnstate Hospital, Arnhem, Netherlands, 4Maasstad Hospital, Rotterdam, Netherlands, 5Leeuwarden Hospital, Leeuwarden, Netherlands, 6HIV Vereniging Nederland, Amsterdam, Netherlands.

Background: After SARS-CoV-2 reached the Netherlands in February 2020, rapid interventions were taken to mitigate viral spread and optimise care for COVID-19 patients. Lockdowns and downscaling of regular healthcare practices were necessary to scale up COVID-19-related care. The effect of these interventions on HIV care are uncertain. We assessed the impact of the nationwide lockdown in March and May during the first COVID-19 wave on HIV diagnosis and linkage to care.

Methods: An observational study was conducted at the Erasmus MC, a regional reference tertiary hospital in the Netherlands. All patients ≥ 18 years presenting with HIV indicator conditions (ICs) were identified in electronic patient records, using an automated identification system for ICD-10 and health insurance codes. Primary outcomes measured were the number of HIV tests performed, number of HIV ICs and corresponding HIV testing rates, and new HIV diagnoses before, during and after lockdown.

Results: From January to April, all newly registered diagnoses decreased by 35%, and in patients referred for HIV ICs by 69% (figure 1). The proportion of patients presenting with HIV ICs that were adequately tested for HIV remained relatively stable, especially where HIV testing is standardised, even during lockdown in March, April and May when a cumulative 328 proven or suspected COVID-19 patients were admitted. The absolute number of HIV tests performed during the first half year of 2020 was 13% lower than the same period in 2019, and new HIV patient referrals dropped 67%. The number of HIV IC, HIV testing rates and HIV referrals showed recovery after the lockdown.

Conclusion: The first two pillars of the HIV care continuum were affected by the lockdown during the COVID-19 pandemic. Standardisation of HIV testing could prevent pandemic delays to a certain extent. With an eye on subsequent COVID-19 waves, these data indicate that maintaining focus on adequate identification and testing of patients with undiagnosed HIV is essential to prevent unwanted declines affecting the 95-95-95 goals.

TRENDS IN TRUVADA AND DESCOVY PRESCRIPTIONS FOR PrEP IN THE UNITED STATES, 2014-2020

Karen W. Hoover1, Weiming Zhu1, Jeffrey Wiener3, Ya-Lin A. Huang1

1Center for Disease Control and Prevention, Atlanta, GA, USA

Background: Emtricitabine and tenofovir disoproxil fumarate (F/TDF) was the only PrEP drug available in the United States until the FDA approved emtricitabine and tenofovir alafenamide (F/TAF) for PrEP on October 3, 2019. Both drugs are safe and effective for PrEP. In 2018, the U.S. healthcare system spent $2.1 billion on PrEP for 18% of persons with a PrEP indication. Less expensive generic formulations of F/TDF are expected in late 2021 when emtricitabine’s patent expires. We estimated trends in the use of these drugs and switching from F/TDF to F/TAF.

Methods: We analyzed data from the IQVIA prescription database to estimate the number of persons prescribed F/TDF or F/TAF for PrEP by calendar quarter from January 2014 through June 2020. During October 1, 2019 through June 30, 2020, we estimated the proportion of new PrEP users prescribed F/TDF or F/TAF. Among a cohort of persons prescribed F/TDF for PrEP by October 3, 2019 and with at least one PrEP prescription after that date, we assessed the proportion who switched to F/TAF. Using a multivariable Poisson regression model, we estimated the probability of switching from F/TDF to F/TAF vs. continuing on F/TDF for PrEP by patient demographic characteristics.

Results: The number of PrEP users prescribed F/TAF increased from 2,637 in the third quarter (Q3) of 2019 to 75,979 in the second quarter of 2020. The number of PrEP users prescribed F/TDF decreased starting in Q1 2019 (Figure). During October 1, 2019 to June 30, 2020, 43,316 (38.1%) of 113,559 new PrEP users were prescribed F/TAF. Among a cohort of 205,248 persons prescribed F/TDF before October 3, 2019 and with at least one PrEP prescription after that date, 57,059 (27.2%) switched to F/TAF. In a multivariable regression model, the adjusted probability of switching from F/TDF to F/TAF vs. continuing on F/TDF for PrEP was 1.49 (95% CI: 1.47-1.51) for persons living in the South vs. the Northeast (aRR 1.52, 95% CI 1.49 – 1.55), persons privately insured vs. publicly insured (aRR 1.29, 95% CI 1.26 – 1.32), and persons living in the South vs. the Northeast (aRR 1.52, 95% CI 1.49 — 1.55).

Conclusion: Since approval of F/TAF in early October 2019, many PrEP users have initiated F/TAF or switched from F/TDF to F/TAF. As new patented and generic PrEP drugs become available, monitoring their use can help understand implications for U.S. healthcare system expenditures. Clinicians might consider prescribing less expensive options that can result in lower healthcare system expenditures for PrEP.

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**Pre-Exposure Prophylaxis (PrEP)** is available since 2016 in France and was highly effective in clinical trials, decreasing HIV incidence to 0.19 per 100 person-years (95% CI 0.11–0.27) among MSM users. HIV testing was recommended prior to PrEP initiation, one month after PrEP initiation and quarterly thereafter. This study aimed to assess the uptake of these guidelines and estimate the incidence of HIV infections among oral PrEP users, by developing an automated surveillance model using the French national health database (Système National des Données de Santé, SNDS).

**Methods:** Using SNDS database, a 3-year historic cohort study included every adult person covered by national health insurance who started an oral PrEP between January 1, 2016 and June 30, 2018. HIV infection was tracked in the follow-up, from the inclusion (1st PrEP dispensation) up to 2019, based on an algorithm including antiretroviral drug deliveries (out of tenofovir-emtricitabine association), laboratory tests for HIV diagnosis or monitoring (results not available), hospitalization data and HIV long-term chronic disease registration. Timelines leading to contamination were reviewed blindly by 2 HIV experts. Risk factors of low adherence to HIV testing in PrEP follow-up were analysed using a generalized linear mixed model.

**Results:** 9,893 PrEP users (99% males, median age 36 (IQR: 30-44) at PrEP initiation) were followed for a median duration of 546 days (IQR: 346-767) with a median of 9 PrEP dispensations (IQR: 4-14). The first HIV test at one month after PrEP initiation was performed by 70% of users. For subsequent tests, this rate exceeded 85% and remained stable over time. HIV testing was lower among PrEP users without prescription refill (OR 0.15, 95% CI 0.12-0.20), but higher if the last prescription was made by a hospital practitioner (OR at 6 month 2.37, 1.88-3.01). After review, 29 HIV infections were identified (Cohen’s kappa = 0.69, 95% CI 0.60-0.76). Among those who did not test for HIV infection had a median duration of 180 days (IQR: 124-490).

**Conclusion:** In addition to clinical research, SNDS could be a powerful automated tool for optimizing PrEP monitoring and identifying risk factors of HIV infection. We confirmed the good follow-up and efficacy of PrEP in users, which should help decreasing HIV incidence in France.

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**A Novel Approach to Measure PrEP Uptake Among Populations with Persistent HIV Risk**

Preeti Pathela1, Saba Qasmi1, Monica Gandhi1, Elliot Rozen1, Harris Goldstein1, Hideaki Okochi1, Karl Stefic1, Leslie Grammatico-Guillon1, Denis Nash1, Marc-Florent Tassi2, Emeline Laurent2, Guillaume Gras3, Lot Florence3, Francis Barni3, Kelly Jamison2, Monica Gandhi1, Amy S. Nunn1, Guillaume Gras1, William C. Goedel1, Saba Qasmieh3, Addie Crawley3

1Centre Hospitalier Universitaire, Tours, France, 2Centre Publique France, Saint-Maurice, France, 3Institut National de la Santé et de la Recherche Médicale, Tours, France

**Background:** Oral pre-exposure prophylaxis (PrEP) is available since 2016 in France and was highly effective in clinical trials, decreasing HIV incidence to 0.19 per 100 person-years (95% CI 0.11–0.27) among MSM users. HIV testing was recommended prior to PrEP initiation, one month after PrEP initiation and quarterly thereafter. This study aimed to assess the uptake of these guidelines and estimate the incidence of HIV infections among oral PrEP users, by developing an automated surveillance model using the French national health database (Système National des Données de Santé, SNDS).

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**Results:** 9,893 PrEP users (99% males, median age 36 (IQR: 30-44) at PrEP initiation) were followed for a median duration of 546 days (IQR: 346-767) with a median of 9 PrEP dispensations (IQR: 4-14). The first HIV test at one month after PrEP initiation was performed by 70% of users. For subsequent tests, this rate exceeded 85% and remained stable over time. HIV testing was lower among PrEP users without prescription refill (OR 0.15, 95% CI 0.12-0.20), but higher if the last prescription was made by a hospital practitioner (OR at 6 month 2.37, 1.88-3.01). After review, 29 HIV infections were identified (Cohen’s kappa = 0.69, 95% CI 0.60-0.76). Among those who did not test for HIV infection had a median duration of 180 days (IQR: 124-490).

**Conclusion:** In addition to clinical research, SNDS could be a powerful automated tool for optimizing PrEP monitoring and identifying risk factors of HIV infection. We confirmed the good follow-up and efficacy of PrEP in users, which should help decreasing HIV incidence in France.

White patients (45%) and MSM with ES (44%), and lowest among Black patients (20%) and women (2%). Agreement between LC-MS and self-reported PrEP use was 91%. Among MSM with rectal CT/GC or ES, PrEP use was associated with age (adjusted prevalence ratio (aPR)=1.6 (95% CI, 1.0-2.5) for ages 25-34 and aPR=2.0 (1.2-3.4) for ages 35-44, vs. 15-24 years); number of recent sex partners (aPR=2.1 (1.3-3.4) for 6-10 partners and aPR=2.0 (1.2-3.3) for >10 partners, vs. <2 partners); having sex/needle-sharing partners with HIV (aPR=1.4 (1.0-1.8)); and inconsistently vs. always using condoms (aPR=3.1 (1.5-6.3)). Race/ethnicity and past-year history of CT/GC/ES diagnoses or post-exposure prophylaxis were not associated.

**Conclusion:** This is the first study to analyze routinely collected remnant samples from STI clinics for a PrEP biomarker. Although the accuracy of self-reported PrEP was high, only 1 in 3 people with a newly diagnosed STI was on PrEP. PrEP use was associated with most measured HIV risk factors, but it is critical to increase use in racial minority populations and women. Surveillance studies using remnant samples can assess the accuracy of self-reported PrEP use in other settings and evaluate the success of interventions to increase PrEP uptake in high-risk populations.

700  

**Pharmacy Reversals: A Novel Indicator of Gaps in the HIV PrEP Care Cascade**

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**Background:** HIV PrEP retention in care is suboptimal with only 50-60% of patients retained in care at 12 months. Common biometric metrics for assessing PrEP persistence may be expensive, inflexible, or burdensome for patients to report in real-time. We introduce PrEP reversals, defined as when patients fail to pick up PrEP prescriptions, as a novel metric for population-based HIV PrEP persistence and a real-time indicator of gaps in the HIV care cascade.

**Methods:** We used a national claims database with up to 75% of all PrEP prescriptions in the United States. Data included claims from October 1, 2015 to September 30, 2019 across all public and private pharmacy types and across all US states. Patients were the unit of analysis. After using a multi-step process to identify HIV PrEP claims, we calculated the percentage of total index HIV PrEP insurer-approved prescription claims that were reversed (i.e. not picked up by the patient and claim withdrawn by the pharmacy). Among those with an initial reversal, we estimated the proportion who delayed (picked up within 90 days), partially abandoned (picked up between day 90 and day 365), or completely abandoned (reversed and not picked up within 365 days) their PrEP prescription over a 12-month period. For each metric, we calculated the percentage of PrEP patients who were later diagnosed with HIV.

**Results:** In our sample of 91,588 patients with 12-months of follow-up data, 14.5% had their index prescription reversed. Of these, 24.9% delayed initiation. Of those not picking up within 90 days, 12.3% filled PrEP between day 90 and day 365 whereas 87.3% did not fill any PrEP. Those who picked up after an initial reversal took an average of 194 days. Among those who completely abandoned their PrEP, 5.7% were diagnosed with HIV – nearly 3 times higher than those who picked up a prescription at some point.

**Conclusion:** Nearly 15% of patients do not pick up their PrEP from the pharmacy, and are at risk of being lost to PrEP care. Roughly two-thirds of patients who reversed their initial prescription ended up not picking up a prescription within 365 days, leaving them at greater risk of HIV. PrEP reversals give a national “lower bound” estimate of PrEP persistence using real-world and real-time data. This novel metric can be used for population-based surveillance, as a marker of those in need of HIV risk reduction intervention, or as an outcome for pharmacy-based interventions to improve PrEP persistence and reduce HIV risk.
701 LOW PROPORTIONS OF LINKAGE & PRESCRIPTIONS OF PrEP IN BLACK WOMEN (THRIVE, 2015-2020)
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Background: To reach the goal of ending the HIV epidemic in the United States, it is important to understand HIV prevention efforts for Black women who continue to be disproportionately affected by HIV, accounting for 58% of new HIV diagnoses among women in 2018. Although perceived behavioral risk is low in Black women, rates of bacterial sexually transmitted infections (STIs), such as syphilis and gonorrhea remain 4.7 times and 6.9 times the reported rate among White women. Those two bacterial STIs are indications for pre-exposure prophylaxis (PrEP), yet PrEP awareness and uptake remains low among Black women. The THRIVE demonstration project supported 7 U.S. health departments to provide comprehensive HIV prevention and care services to men who have sex with men of color but did not exclude other clients from receiving services.

Methods: We analyzed preliminary data collected from 8,648 non-Hispanic Black women enrolled in the THRIVE demonstration project. Data were reported by 7 project sites located in Alabama, Baltimore, Louisiana, New York City, Philadelphia, Virginia, and Washington DC from September 2015 through June 2020. We estimated the proportion of Black women aged ≥18 years who received HIV testing, STI screening, and PrEP services (e.g., screened, referred and linked to a PrEP provider, and prescribed PrEP) and calculated the positivity rates of syphilis, gonorrhea, and chlamydia.

Results: Among the 7,137 Black women in the THRIVE demonstration project who were HIV negative, 2,702 (38%) were eligible for PrEP and 2,488 (35%) were referred to a PrEP provider, yet only 217 (3%) were linked with a PrEP provider and 142 (2%) were prescribed PrEP medication (see Figure 1). Among all Black women in the sample, 69.8% were screened for bacterial STIs, including 62.9% for syphilis, 66.0% for gonorrhea, and 65.6% for chlamydia. The positivity rates of STI tests were 3.2% for syphilis, 4.6% for gonorrhea, and 4.8% for chlamydia. Between Black women in the sample, 69.8% were screened for bacterial STIs, including 62.9% for syphilis, 66.0% for gonorrhea, and 65.6% for chlamydia. Among Black women who were eligible, the proportions who were linked to a PrEP provider and prescribed PrEP were extremely low. In order to reach the goal of ending the HIV Epidemic in the United States, it is imperative that Black women have access to PrEP information and care. Programmatic activities focused on specifically meeting the HIV prevention needs of Black women are greatly needed.

Conclusion: Among Black women who were eligible, the proportions who were linked to a PrEP provider and prescribed PrEP were extremely low. In order to reach the goal of ending the HIV Epidemic in the United States, it is imperative that Black women have access to PrEP information and care. Programmatic activities focused on specifically meeting the HIV prevention needs of Black women are greatly needed.

702 PrEP USE AND REFERRAL: BLACK PARTNERS OF PEOPLE WITH HIV IN PARTNER SERVICES, 2019
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1Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Blacks or African Americans (hereafter Blacks) are disproportionately affected by Human Immunodeficiency Virus (HIV) in the United States. Effective prevention strategies must be scaled up in Black communities to achieve the national goal of reducing HIV-related disparities. HIV partner services (PS) provide an opportunity to link HIV-negative partners to prevention services, including pre-exposure prophylaxis (PrEP). However, the extent to which PrEP services are integrated into PS programs is not well known. This analysis examines current use of PrEP and referral to PrEP providers among HIV-negative Black partners contacted by PS programs at CDC-funded health departments.

Methods: In 2019, 48 of 60 health departments reported data on current PrEP use or referral to PrEP providers among HIV-negative partners of people with HIV (PWH). Of these, 20 reported PrEP data for ≥70% of HIV-negative partners and were used in this analysis. We conducted descriptive analysis to examine the pattern of current PrEP use and referral to PrEP providers among Black partners by age, gender, and U.S. census region. In addition, we conducted multivariate Poisson regression analyses to estimate the independent associations of age, gender, and U.S. census region with PrEP use and referral among Blacks. Adjusted prevalence ratios (aPR) and 95% confidence intervals (CI) were calculated.

Results: Partner services identified 710 HIV-negative Black partners of PWH. Of these, only 52 (7.3%) reported taking PrEP at the time of their contact with PS. There were no significant differences in prevalence of current PrEP use by age, gender, and geographic region. PS offered referral to 251 of 608 (41.3%) HIV-negative Black partners who were not on PrEP. There were no significant variations in PrEP referral by age and gender. However, Black partners residing in the South were less likely (14.7%; aPR = 0.25, 95% CI = 0.18–0.36) and those in Midwest more likely (70.4%; PR = 1.23, 95% CI = 1.03–1.47) than those in Northeast (55.4%) to have been referred to PrEP providers.

Conclusion: Less than one-half of Black partners of PWH contacted by PS were currently taking PrEP or referred to PrEP providers, suggesting continued risk for HIV infection. Low levels of PrEP use in general, and lower levels of PrEP referral in South indicate that PS programs need to identify and remove barriers to scale-up PrEP services among Blacks at risk for HIV infection.

Table 1. Number and percent of Black partners of people with HIV who were on PrEP or referred to PrEP providers by demographic characteristics, 20 health departments, 2019

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Total HIV - Negative Partners</th>
<th>Total HIV - Negative Partners Not on PrEP by Partner Services</th>
<th>Total HIV - Negative Partners Referred to PrEP Providers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (Est. %)</td>
<td>n (Est. %) aPR (95% CI)</td>
<td>n (Est. %) aPR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>329 (96.7)</td>
<td>329 (96.7)</td>
<td>329 (96.7)</td>
</tr>
<tr>
<td>30-49</td>
<td>251 (88.6)</td>
<td>251 (88.6)</td>
<td>251 (88.6)</td>
</tr>
<tr>
<td>50+</td>
<td>70 (76.6)</td>
<td>70 (76.6)</td>
<td>70 (76.6)</td>
</tr>
<tr>
<td>Male Gender</td>
<td>496 (99.9)</td>
<td>496 (99.9)</td>
<td>496 (99.9)</td>
</tr>
<tr>
<td>Female Gender</td>
<td>100 (98.7)</td>
<td>100 (98.7)</td>
<td>100 (98.7)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>236 (96.1)</td>
<td>236 (96.1)</td>
<td>236 (96.1)</td>
</tr>
<tr>
<td>Other</td>
<td>62 (79.2)</td>
<td>62 (79.2)</td>
<td>62 (79.2)</td>
</tr>
<tr>
<td>Census Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>205 (95.2)</td>
<td>205 (95.2)</td>
<td>205 (95.2)</td>
</tr>
<tr>
<td>Midwest</td>
<td>35 (94.3)</td>
<td>35 (94.3)</td>
<td>35 (94.3)</td>
</tr>
<tr>
<td>South</td>
<td>145 (96.5)</td>
<td>145 (96.5)</td>
<td>145 (96.5)</td>
</tr>
<tr>
<td>Total</td>
<td>732 (99.8)</td>
<td>732 (99.8)</td>
<td>732 (99.8)</td>
</tr>
</tbody>
</table>

Note: aPR = adjusted prevalence ratio; CI = confidence interval. PWH = people with HIV.

**Data for partners with missing age (6.7%) and gender (4.3%) are not shown on the table.**

**Total number (n = 608) excludes HIV-negative partners already on PrEP (n = 52; 7.3%) and those who had a missing value on the variable "Referred to PrEP Providers" (n = 90). Data for partners with missing age (n = 64; 10.3%) and gender (n = 60; 10.3%) are not shown on the table.**

703 PREEXPOSURE PROPHYLAXIS TRENDS BY HEALTH CENTER FEDERAL FUNDING STATUS, 2014-2019
Kirk D. Henny1, Weiming Zhu2, Patrick Schoen3, Ya-Lin A. Huang3, Lei Yu3, Suma Nair1, Karen W. Hoover1
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Health Resources and Services Administration, Rockville, MD, USA, 3IIEF International, Atlanta, GA, USA

Background: Expanding access to preexposure prophylaxis (PrEP) is an important strategy for achieving the goals of the Ending the HIV Epidemic
initiative (EHE), particularly in high HIV burden urban areas. Federally Qualified Health Centers (FQHCs) and other healthcare settings serve a critical role in implementing HIV prevention strategies in EHE Phase I jurisdictions, where HIV diagnoses comprise more than half of annual HIV diagnoses. We assessed the trends in number of persons prescribed PrEP and the number of PrEP providers in the 48 EHE phase I urban jurisdictions.

Methods: We identified PrEP users and their PrEP providers in the IQVIA Real-World Longitudinal Prescription database during 2014-2019 using a previously validated algorithm. We identified all persons who were prescribed PrEP in EHE Phase I urban jurisdictions. Provider locations in the IQVIA database were cross-referenced with the Health Resources and Services Administration (HRSA) administrative and Uniform Data System data to distinguish between HRSA supported FQHC and non-FQHC providers. We estimated the annual numbers of PrEP users and PrEP providers among 877 HRSA supported FQHCs and 86,636 non-FQHC healthcare locations. We used a Poisson regression model to calculate the estimated annual percentage change (EAPC) and confidence intervals (CI) in the number of PrEP users and PrEP providers during the study period.

Results: The overall number of PrEP users in Phase I urban jurisdictions increased from 1,656 in 2014 to 27,479 in 2019 (EAPC 59.5, [95% CI 57.8, 60.3]). The number of PrEP users in HRSA FQHCs (EAPC 101.5 [95% CI 97.6, 105.5]) increased more than in non-FQHCs (EAPC 56.2 [95% CI 55.4, 57.0]) (Table 1). The overall number of PrEP providers in Phase I urban jurisdictions increased from 586 in 2014 to 3,635 in 2019 (EAPC 38.4 [95% CI 36.6, 40.0]). The number of PrEP providers increased more in HRSA FQHCs (EAPC 49.3 [95% CI 41.9, 56.7]) compared to non-FQHCs (EAPC 37.8 [95% CI 36.2, 39.4]) (Table 1). Providers in HRSA FQHCs also had higher average PrEP patient per provider volume in 2019 (16.4 [95% CI 9.2, 23.5]) compared to those in non-FQHCs (7.0 [95% CI 6.2, 8.6]).

Conclusion: The growing numbers of PrEP users and providers highlight increasing access to and use of PrEP in EHE urban jurisdictions. The methods developed in our study can be used to evaluate local and national PrEP implementation activities in HRSA supported FQHCs in EHE jurisdictions.

**Table 1: Summary of cross-sectional population-based surveys (N/IP) and PrEP providers in EHE urban jurisdictions by HRSA-supported FQHCs (ten states, United States, 2014-2019)**

<table>
<thead>
<tr>
<th>Group</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>2019 EAPC (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrEP Users</td>
<td>51</td>
<td>210</td>
<td>430</td>
<td>870</td>
<td>1,412</td>
<td>2,021</td>
<td>10.1 (9.7, 10.5)</td>
</tr>
<tr>
<td>non-FQHC</td>
<td>1,077</td>
<td>4,229</td>
<td>7,071</td>
<td>11,754</td>
<td>20,011</td>
<td>25,814</td>
<td>5.6 (5.2, 6.0)</td>
</tr>
<tr>
<td>PrEP Providers</td>
<td>51</td>
<td>210</td>
<td>430</td>
<td>870</td>
<td>1,412</td>
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</tr>
<tr>
<td>non-FQHC</td>
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<td>11,754</td>
<td>20,011</td>
<td>25,814</td>
<td>5.6 (5.2, 6.0)</td>
</tr>
</tbody>
</table>

**704 OPTIMIZING HIV PREVENTION EFFORTS TO ACHIEVE EHE INCIDENCE TARGETS**

*Evvin Jacobson1, Katherine A. Hicks2, Justin Carrico1, David Purcell1, Timothy Green1, Jonathan Mermin1, Paul Farnham1*

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Background: We optimized societal spending on HIV prevention (increased by given fixed amounts of federal funds) to assess whether it is possible to decrease annual HIV incidence in the United States to less than 10,000 cases in 5 years and less than 3,000 cases in 10 years, and thus achieve the HHS Ending the Epidemic (EHE) incidence targets.

Methods: We applied the HIV Optimization and Prevention Economics (HOPE) model, a dynamic, compartmental model that simulates that portion of the U.S. population aged ≥13 that is sexually active or injects drugs. Our analytic time horizon was 2020 through 2029. The model applied current estimated public and private HIV prevention spending ($2.803 billion for 2020) each year to the following intervention categories: HIV screening (MSM and heterosexuals at high and at low risk, and PWID), HIV care continuum (linkage to care and after diagnosis, prescription of ART, retention in care, viral suppression), PrEP, and SSPs. To model the effect of additional prevention funding, we divided the 10-year time frame into three time periods and added $500M/year for 2020-2021, $1.5B/year for 2022-2024, and $2.5B/year for 2025-2029. Using these scenarios, we estimated the impact of additional prevention and treatment spending with and without optimizing allocation of funds to the most impactful interventions: Scenario 1a with no optimization; Scenario 1b where the optimization started in year 6 of EHE period (2025, phase 2 of EHE); and Scenario 1c where the optimization started in year 3 of EHE period (2022).

Results: The additional prevention and treatment spending was approximately $15B higher over the 10-year time period in all scenarios compared to the current allocation, and total infections decreased by around 190,000 to 240,000 in the three scenarios compared to the current allocation (Table: Scenario Comparisons). Only in Scenario 1c did the allocation of funds allow the 2024 and 2029 incidence targets to be met.

Conclusion: All three scenarios resulted in dramatic decreases in HIV incidence. However, optimization of prevention funding early in the time period was needed to reach EHE targets. An optimal allocation of resources is difficult to achieve in the real world, as it assumes flexibility of funding between various governmental and private agencies and programs to maximize efficacy of available funding. The EHE initiative has the potential for reaching ambitious goals with the dedication of significant funding increases across all 10 years of the initiative.

**Table: Scenario Comparisons**

<table>
<thead>
<tr>
<th>Scenario</th>
<th>1a</th>
<th>1b</th>
<th>1c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incidence</strong></td>
<td>363,894</td>
<td>174,757</td>
<td>150,982</td>
</tr>
<tr>
<td><strong>Decrease in new infections compared to baseline</strong></td>
<td>186,717</td>
<td>220,311</td>
<td>279,881</td>
</tr>
<tr>
<td><strong>Prevention funding ($M)</strong></td>
<td>28,020</td>
<td>46,020</td>
<td>46,020</td>
</tr>
<tr>
<td><strong>Treatment spending ($M)</strong></td>
<td>333,847</td>
<td>332,572</td>
<td>330,123</td>
</tr>
<tr>
<td><strong>Prevention and treatment spending ($M)</strong></td>
<td>361,867</td>
<td>378,592</td>
<td>376,053</td>
</tr>
<tr>
<td><strong>Additional prevention and treatment spending compared to baseline ($M)</strong></td>
<td>17,224</td>
<td>14,976</td>
<td>10,667</td>
</tr>
<tr>
<td><strong>2034 annual incidence (target 50)</strong></td>
<td>35,313</td>
<td>48,427</td>
<td>48,412</td>
</tr>
<tr>
<td><strong>2039 annual incidence (target 3)</strong></td>
<td>38,025</td>
<td>7,614</td>
<td>3,154</td>
</tr>
</tbody>
</table>

* Annual societal HIV prevention funding: $2.803B
** Calculated by multiplying the total annual prevention funding by the number of years at that funding level for years 1 to 10.
*** Treatment spending, an outcome of the simulation, is dependent on the number of people on ART for each scenario.
1 No optimization
2 Optimization starting in year 6 (2025, phase 2 of EHE)
3 Optimization starting in year 3 of EHE (2022)

**705 PREDICTORS OF PrEP UPTAKE IN A SEXUAL HEALTH CLINIC WITH IMMEDIATE PrEP INITIATION**

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1University of California San Diego, San Diego CA, USA

Background: Improved HIV pre-exposure prophylaxis (PrEP) uptake will be necessary for HIV eradication initiatives. Offering PrEP at the time of HIV testing can improve uptake by avoiding delays between HIV screening and initiation of PrEP typical in the traditional clinic setting. We instituted an immediate PrEP initiation program and assessed predictors of PrEP interest, initiation, and linkage.

Methods: Between November 2018 and February 2020, PrEP-eligible individuals who presented to a community-based sexual health clinic in San Diego, California were assessed for interest in immediate PrEP initiation (I-PrEP). Interested individuals were referred to a study pharmacist to receive a free 30-day supply of PrEP as early as same day and within 7 days of HIV testing, and were also linked to a community PrEP care provider. Demographic, behavioral, and sexually transmitted infection (STI) data were collected. Univariable and multivariable analyses were conducted to determine predictors of PrEP interest, initiation, linkage, and retention in care at 3 months.

Results: Out of 2,349 individuals who presented for HIV/STI testing, 1,348 were eligible for PrEP, out of whom 517 (38.4%) were interested in starting PrEP and referred to the study pharmacist. Among those referred to the pharmacist, 333 (24.7%) actually started PrEP, 278 (20.6%) were linked to PrEP care, and, among those with follow-up, 78 (5.6%) remained in care at 3 months (Figure). Among predictors of multiple PrEP outcomes: testing positive for gonorrhea predicted PrEP interest (aOR 2.44; 95% CI 1.48-4.02), initiation (aOR 5.00; 95% CI 2.20-11.39), and linkage (aOR 2.31; 95% CI 1.26-4.25). Non-Black race predicted both PrEP initiation (aOR Black 0.50: 95%CI 0.27-0.95) and linkage (aOR Black 0.32: 95%CI 0.16-0.64). Having private health insurance predicted both linkage (aOR 1.85: 95% CI 1.28-2.67) and retention (aOR 3.94: 95% CI 1.77-8.75).

Conclusion: Immediate PrEP initiation in a sexual health clinic was feasible, although only a minority of PrEP-eligible persons initiated PrEP and remained...
in care at 3 months. Having gonorrhea was a strong predictor of PrEP uptake. Being non-Black race and having private health insurance also predicted PrEP uptake, consistent with racial/ethnic and socioeconomic barriers to PrEP usage. Greater support is needed at each step of the PrEP intervention continuum to improve the implementation of similar programs.

706 THE M-CUBED APP TO IMPROVE HIV PREVENTION AND CARE OUTCOMES IN MSM: RESULTS OF AN RCT

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Emory University, Atlanta, GA, USA, University of Michigan, Ann Arbor, MI, USA, State University of New York Downstate Medical Center, Brooklyn, NY, USA, University of Pennsylvania, Philadelphia, PA, USA, Columbia University, New York, NY, USA, City University of New York, New York, NY, USA, US Centers for Disease Control and Prevention, Atlanta, GA, USA, San Diego State University, San Diego, CA, USA, University of New York at Albany, Rensselaer, NY, USA

Background: Gay, bisexual and other men who have sex with men (GBMSM) face the highest burden of HIV in the United States, and there is a paucity of efficacious mobile health HIV prevention and care interventions tailored for diverse risk profiles of GBMSM. We developed and tested a mobile app (M-Cubed) that combined prevention messages and access to core prevention services among GBMSM in three US cities to promote key health services for HIV-negative MSM (HIV testing, STI testing, PrEP), and MSM living with HIV (HIV care, STI testing, condom use) and MSM living with HIV (HIV care, STI testing, condom use).

Methods: GBMSM (in three groups: lower-risk HIV-negative, higher-risk HIV-negative, and living with HIV) from Atlanta, Detroit, and New York City were randomized to a waitlist control. Men allocated to the intervention arm could access the M-Cubed app to improve the implementation of similar programs.

707 RISK-BASED VS UNIVERSAL PrEP DELIVERY DURING PREGNANCY: A CLUSTER RANDOMIZED TRIAL

John Kinuthia1, Julia Dettinger2, Joshua Stein3, Nancy Mwongeli4, Laureen Gomez2, Felix Abuna1, Ben Ochieng5, Salpine Wawatty1, Mary Marwa6, Anjuli Wagner7, Barbra A. Richardson7, Jillian Pintye1, Jared Baeten8, Grace John-Stewart1

1Kenyatta National Hospital, Nairobi, Kenya, 2University of Washington, Seattle, WA, USA

Background: PrEP provision in maternal child health (MCH) clinics is important for women at risk for HIV acquisition during pregnancy. In high prevalence settings, the best strategies for PrEP delivery that balance benefits of HIV prevention against unnecessary PrEP exposure are unknown.

Methods: The PrEP Implementation for Mothers in Antenatal Care (PRIMA) cluster randomized trial compared two models of PrEP delivery in MCH clinics (NCT03070600). Twenty facilities in Siaya and Homa Bay, Kenya were randomized to either Targeted PrEP (HIV risk assessment via validated HIV risk score, and HIV self-test provision for partners, with PrEP offered to those with high-risk or if requested) versus Universal PrEP (standardized counseling and PrEP offer to all). The Targeted approach was hypothesized to improve risk perception and PrEP decision-making. Participants were enrolled during pregnancy and followed to 9 months postpartum. Primary outcomes were HIV incidence and ‘appropriate PrEP use’ (defined as PrEP used by women with high risk and not used by women with low risk per HIV risk score). PrEP was prescribed per national guidelines. Outcomes were compared between arms, clustered on facility and adjusted for baseline differences using generalized estimating equations.

Results: Between January 2018 and July 2019, 4,434 pregnant women were enrolled (2,197 Targeted, 2,250 Universal). Median age was 24 years (IQR 21, 28), most (85%) were married, and median gestational age was 24 weeks (IQR 20, 30). Overall, 1,877 (42%) were at risk for HIV acquisition at baseline, greater in the Targeted group (51% vs 33%, p<0.001). Retention at 9 months postpartum was 94% (92% Targeted, 96% Universal). PrEP was accepted by 18% of women in the Targeted arm versus 20% of women in the Universal arm (adjusted Risk Ratio 1.1; 95% CI 0.9-1.4). Median duration of PrEP use was similar (8.9 vs 8.6 months, p=0.9). HIV incidence was 0.3 and 0.4/100 py (aRR 0.7, 95% CI 0.2-2.1).

Conclusion: At MCH sites in Kenya, a substantial proportion of pregnant women were at risk for HIV, used and continued PrEP, and maternal HIV incidence was low. Targeting by risk-based PrEP offer did not improve PrEP decision-making or decrease HIV incidence. Offering Universal PrEP counselling is an effective and efficient approach to achieve appropriate PrEP use among women at risk.

Table 1: PRIMA study outcomes

<table>
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<tr>
<th></th>
<th>All subjects (N=4444)</th>
<th>% (N=4444)</th>
<th>% (N=2950)</th>
<th>% (N=2950)</th>
<th>% (N=2950)</th>
</tr>
</thead>
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<tr>
<td>Targeted arm</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>HIV incidence</td>
<td>0.3 (0.2-0.6)</td>
<td>0.3 (0.1-0.6)</td>
<td>0.4 (0.2-0.7)</td>
<td>0.7 (0.3-1.1)</td>
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<td>PrEP acceptance</td>
<td>828 (18.8)</td>
<td>397 (17.6)</td>
<td>441 (18.6)</td>
<td>0.6 (0.4-1.1)</td>
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<tr>
<td>Any PrEP use</td>
<td>722 (16.3)</td>
<td>326 (14.8)</td>
<td>397 (17.6)</td>
<td>0.6 (0.4-1.0)</td>
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<tr>
<td>Continued PrEP to 9 months postpartum (n=723)</td>
<td>387 (51)</td>
<td>167 (51)</td>
<td>200 (50)</td>
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<td></td>
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<tr>
<td>PrEP duration (months)</td>
<td>8.8 (3.3-11.1)</td>
<td>8.9 (3.7-11.9)</td>
<td>8.8 (3.3-11.4)</td>
<td>p=0.9</td>
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708 GLOBAL AND REGIONAL ESTIMATES OF THE CONTRIBUTION OF HSV-2 TO INCIDENT HIV INFECTIONS

Romain Silhol, Helen Coupland, Rebecca Baggsale, Lon Miller, Lisa Staedegaard, Sami Lynne Gottlieb, James Stannah, Katherine M. Turner, Peter Vickermax, Richard Hayes, Philippe Mayaud, Katharine J. Looker, Marie-Claude Bolly

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Background: Evidence of biological interactions between herpes simplex virus type 2 (HSV-2) and HIV suggests that HSV-2 plays an important role in HIV epidemics. We improved previous estimates of the contribution of HSV-2 to incident HIV infections using a dynamic-transmission model accounting for direct/indirect transmissions (using population-attributable fractions (PAFs)).

Methods: We developed a mathematical model of HSV-2/HIV transmission among 15-49-year-old heterosexual, non-drug injecting populations, calibrated
to each of the six WHO regions using region-specific demographic and HSV-2/ HIV epidemiological data. We derived global and regional estimates of the 10-year tPAF under three additive scenarios, assuming: (1) HSV-2 only increases HIV acquisition ("conservative" scenario), (2) HSV-2 increases HIV acquisition and transmission ("liberal"), and (3) HIV/ART (antiretroviral therapy) also modifies HSV-2 transmission and HSV-2 decreases ART effect on HIV transmission ("fully liberal"). We compared predicted numbers of incident HIV infections (over 2009-2018) between each scenario and its counterfactual: no biological interactions.

Results: HSV-2/HIV biological interactions were necessary to reproduce empirical data on HIV incidence by HSV-2 status in Africa. Under the conservative scenario, our model predicted a tPAF of 37.3% (95% uncertainty interval 33.4–43.2%) and 5.6 (4.5–7.0) million incident heterosexual HIV infections due to HSV-2 globally over 2009-2018. The contribution of HSV-2 to incident HIV infections was largest for the African region (42.6% [38.0–51.2%]) and 4.8 (3.6–6.5) million infections, respectively, and lowest for the European region (11.2% [7.9–13.8%]) and 0.11 (0.07–0.15) million (Figure). The tPAF was higher among female sex workers, their clients, and older adults, reflecting their higher HSV-2 prevalence. Under the liberal scenario, the tPAF was 51.0% (42.7–58.2%) globally, 1.3–2.4-fold higher compared to the conservative scenario across regions. Accounting for additional modifying effects between HSV-2 and HIV/ART in the fully liberal scenario did not influence tPAF estimates, and tPAF did not substantially change when calculated over 2019-2028.

Conclusion: Our results suggest that HSV-2 contributed to over a third of new HIV infections worldwide over 2009–2018 and will contribute similarly over 2019–2028. This was highest in Africa, despite increased ART access. Improved HSV-2 control measures, such as future vaccines could have a substantial impact on HIV incidence.

**709 DAILY DOXYCYCLINE IN MSM ON PrEP FOR PREVENTION OF SEXUALLY TRANSMITTED INFECTIONS**

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**Background:** MSM continue to experience high rates of sexually transmitted infections (STI). Use of HIV pre-exposure prophylaxis (PrEP) significantly reduces risk of HIV infection, and a similar strategy using daily doxycycline may serve as STI PrEP. We undertook a pilot study to determine STI outcomes of HIV-negative MSM on dual HIV/STI PrEP.

**Methods:** HIV-negative MSM with prior diagnosis of syphilis received 48 weeks of tenofen DF 300mg-emtricitabine 200mg daily and were randomized 1:1 to receive either immediate daily doxycycline 100mg, or deferred doxycycline beginning 24 weeks later in an open-label pilot study of Dual Daily HIV and Syphilis PrEP (The DuDHS Study) in Vancouver, Canada. Participants underwent screening for STI every 3 months, with Staphylococcus aureus cultures collected to evaluate tetracycline/doxycycline resistance by Kirby Bauer testing. STI rates were compared between those on dual PrEP vs. HIV PrEP alone over the initial 24 weeks using Fishers exact test.

**Results:** Fifty-two MSM were randomized with median age of 34 years (interquartile range [IQR], 29 – 43). Overall, 55.8% self-reported prior gonorrhea and chlamydia infection. Chlamydia infection occurred only in the deferred arm during the first 24 weeks (n=10) (rate 0 vs. 8.16/100 PY, p = 0.001), subsequently no infections occurred in either arm. No individuals in the immediate arm, and one individual in the deferred arm developed syphilis infection during the first 24 weeks (rate 0 vs. 0.81/100 PY, p = 0.98) with no infections seen thereafter in either arm. By 24 weeks, n= 4 in the immediate arm and n=7 in the deferred arm tested positive for gonorrhea (rate 31.37 vs. 57.14/100 PY, p= 0.503), and only one additional infection was seen in each arm for 24 – 48 weeks. In a logistic model receipt of doxycycline was associated with reduced probability of any STI (OR 0.18, 95% CI 0.05 – 0.68) during the first 24 weeks. Tetracycline resistance was seen in 1/5 S. aureus isolates at 24 weeks and 3/6 isolates at 48 weeks in the immediate arm and in 1/2 isolates after six months of doxycycline use in the deferred arm.

**Conclusion:** STI PrEP using daily doxycycline demonstrated decreased rates of chlamydia infection while impact on syphilis could not be ascertained. Tetracycline resistance amongst nasal carriage of S.aureus was observed over the study duration. Further evaluation of potential benefits and antimicrobial resistance in a larger study may be warranted.

**710 TENOFOVIR HAIR LEVELS SIMILAR AMONG PREGNANT AND POSTPARTUM PrEP USERS**

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**Background:** Pregnancy may alter the pharmacokinetics (PK) of PrEP drugs in some biomarkers, with lower tenofovir (TFV) or metabolite concentrations in plasma and dried blood spots observed in pregnancy versus postpartum. Hair TFV levels measure cumulative exposure but have not been used to assess adherence among perinatal PrEP users to date. We measured hair TFV levels among Kenyan PrEP users, during pregnancy and postpartum.

**Methods:** PrEp Implementation for Mothers in Antenatal Care (PrIMA) is a cluster randomized trial in Kenya (NCT03070600) evaluating different PrEP counseling strategies for women in antenatal care. Women enrolled in PrIMA are followed through 9 months postpartum. Hair samples were collected at visits from a subset of women who reported using PrEP in the last 30 days. TFV hair levels in the distal 1 cm of hair (reflecting last 4 weeks of use) were analyzed by a validated liquid chromatography/tandem mass spectrometry assay. Correlates of weight-normalized TFV hair concentrations were identified via linear regression.

**Results:** In total, 164 hair samples were analyzed from 109 women. One-third (32%) of samples were from pregnancy visits and 68% were from postpartum visits. Median age of women was 27 years (IQR 23–34), 30% had partners known to be living with HIV, and median time on PrEP was 6 months (IQR 3–9) at sample collection. Median hair TFV concentration was 0.005 ng/mg (IQR 0.002–0.030) across pregnancy visits and 0.005 ng/mg (IQR 0.002–0.028) across postpartum visits and similar across timepoints (Figure 1). Overall, 29% of samples had TFV levels ≥0.023 mg/ng indicating ≥4 PrEP doses/week, according to benchmarks established in directly observed PK studies among non-pregnant participants; there was no difference pregnancy vs. postpartum in this benchmark (28% vs. 31%, p = 0.68). Pregnancy status was not associated with TFV hair levels (p = 0.59). Having a partner known to be living with HIV was associated with higher TFV levels in both pregnancy (p < 0.006) and postpartum (p < 0.001). Among women with TFV levels available both in pregnancy and postpartum (n = 28), median TFV levels were 0.004 ng/mg (IQR 0.002–0.022) in pregnancy vs. 0.005 ng/mg (IQR 0.002–0.033) postpartum (p = 0.62).

**Conclusion:** Our study shows that TFV levels in hair samples collected from PrEP users were comparable during pregnancy and postpartum. Hair metrics serve as cumulative measure of exposure and are unlikely to need adjustment for PK differences in the perinatal period when used as adherence metrics.
711 THE IMPACT OF VIOLENCE ON PrEP ADHERENCE AMONG US CISGENDER WOMEN AT RISK FOR HIV
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1University of California San Diego, La Jolla, CA, USA; 2University of California Los Angeles, Los Angeles, CA, USA; 3Los Angeles Biomedical Research Institute at Harbor–UCLA Medical Center, Torrance, CA, USA; 4Gilead Sciences, Inc, Foster City, CA, USA; 5University of Michigan, Ann Arbor, MI, USA

Background: Violence is prevalent against women at-risk for HIV; survivors are at increased behavioral risk for HIV, while survivorship is linked to higher HIV susceptibility, via dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and female genital tract (FGT) microenvironment after physical or psychological trauma. Pre-exposure prophylaxis (PrEP) for HIV can mitigate this risk, but adherence remains challenging. We report findings on the impact of violence on PrEP adherence among at-risk cisgender women taking oral PrEP (daily tenofovir disoproxil fumarate/emtricitabine).

Methods: Adherence Enhancement Guided by Individualized Texting and Drug Levels (AEGiS) was an open-label clinical trial of PrEP adherence in cisgender women ≥18 years old at-risk for HIV in Southern California. Adherence was supported by two-way text messaging and counseling based on tenofovir diphosphate (TFV-DP) concentrations in dried blood spots. Participants completed a survey inclusive of violence history at weeks 0, 4, 12, 24, 36, and 48. TFV-DP concentrations consistent with ≥4 doses/week were considered to be “high adherence.” Logistic regressions were used to assess odds of adherence.

Results: Of 136 participants, 38% were non-Hispanic Black, and 19% identified as Latina; mean age was 40 (SD:11). 22% (n=29) reported past year violence; 16% (n=21) reported physical and 15% (n=20) sexual violence. Sexual violence on lifetime was reported by 30% (n=39). Odds of high adherence at four weeks post-PrEP initiation are presented in Table 1; 50, or 43%, of participants completed a survey inclusive of violence history at weeks 0, 4, 12, 24, 36, and 48. TFV-DP concentrations consistent with ≥4 doses/week were considered to be “high adherence.” Logistic regressions were used to assess odds of adherence. 

Conclusion: Cisgender women with experiences of violence have lower odds of having TFV-DP concentrations reflective of high or near-perfect adherence one-month post-initiation, compared to women not reporting violence. Providing support through comprehensive trauma services may improve both adherence and immune functioning. Given the prevalence of violence among women at-risk for HIV, PrEP programs should emphasize trauma screening and care in service delivery.

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Background: Men who have sex with men (MSM) who experience sexual violence (SV) are at increased risk for HIV. Pre-exposure prophylaxis (PrEP) is effective against HIV infection and can protect HIV-negative MSM experiencing SV from HIV acquisition. We estimated the percentage of PrEP use among MSM who reported experiencing SV in the past 12 months and examined the association between SV and PrEP use among MSM overall and by subgroups.

Methods: In 2017, National HIV Behavioral Surveillance (NHBS) used venue-based sampling to recruit and interview MSM in 23 U.S. cities. We analyzed data on key characteristics and SV experiences and PrEP use in the past 12 months among HIV-negative MSM. MSM with likely indications for PrEP included those who had either a male HIV-positive partner or ≥2 male partners and had either an STI or condomless anal sex with a male partner in the past 12 months. Weighted row percentages with 95% confidence intervals (CI) were reported. P-values were calculated using Rao-Scott chi-square tests.

Results: Overall, 7,121 HIV-negative MSM participated. Of these, 5.4% (95% CI: 4.6%-6.6%) reported experiencing SV in the past 12 months. MSM who reported experiencing SV were more likely to use PrEP compared to MSM who did not report experiencing SV (34.9% vs. 25.7%, p=0.008). A higher percentage of MSM who reported experiencing SV likely met the clinical indications for PrEP (82.7% vs. 77.3%, p=0.075). MSM who reported experiencing SV were more likely to use PrEP regardless of age group (18-29 years: 33.6% vs. 24.8%, p=0.037; 30 or older: 36.8% vs. 26.5%, p=0.048). Among white MSM, those who reported experiencing SV were more likely to use PrEP (44.0% vs. 31.7%, p=0.028). Among Hispanic/Latino or Black MSM, those who reported and who did not report experiencing SV had similar percentages of PrEP use (Hispanic/Latino: 29.4% vs. 21.8%, p=0.244; Black: 24.7% vs. 20.4%, p=0.478). MSM who reported experiencing SV were more likely to use PrEP regardless of health insurance coverage (insured: 37.2% vs. 28.7%, p=0.032; uninsured: 24.6% vs. 12.3%, p=0.040) or same-sex discrimination in healthcare (discriminated against: 55.8% vs. 29.1%, p=0.040; not discriminated against: 33.7% vs. 25.6%, p=0.020).

Conclusion: PrEP use was higher among MSM who experienced SV in the past 12 months overall and across multiple subgroups. MSM who experience SV may be more likely to need and initiate PrEP. Clinical SV screening may be an opportunity to identify HIV risk and PrEP needs and to assess MSM’s safety.
713 PREDICTORS OF PrEP ADHERENCE AND RETENTION IN US CIGENDER WOMEN AT RISK FOR HIV


1University of California San Diego, La Jolla, CA, USA, 2University of California San Diego, San Diego, CA, USA, 3University of Michigan, Ann Arbor, MI, USA, 4University of California Los Angeles, Los Angeles, CA, USA, 5Massachusetts General Hospital, Boston, MA, USA, 6Harbor–UCLA Medical Center, Torrance, CA, USA, 7Galead Sciences, Inc, Foster City, CA, USA, 8University of Colorado, Aurora, CO, USA

Purpose: HIV pre-exposure prophylaxis (PrEP) effectiveness depends on adherence, which requires retention in PrEP care. We examined factors associated with PrEP adherence and retention among at-risk cisgender women prescribed oral PrEP.

Methods: Adherence Enhancement Guided by Individualized Texting and Drug Levels (AEGIS) was a 48-week single-arm open-label demonstration study of daily oral tenofovir disoproxil fumarate/emtricitabine in cisgender women ≥18 years old at risk for HIV conducted at five Southern California sites. Study visits occurred at baseline and at weeks 4, 12, 24, 36, and 48. Adherence was supported with text messages and titrated adherence counseling based on rapid-turnaround tenofovir diphosphate (TFV-DP) concentrations from dried blood spots. Adherence was examined in four ways: (a) having any TFV-DP ≥4 doses per week (d/w), (b) having any TFV-DP ≥6 d/w, (c) having all TFV-DP ≥4 d/w, and (d) having all TFV-DP ≥6 d/w at all visits attended. Retention was defined as completing the week 48 visit. We used univariate and multivariable logistic regression to identify baseline demographic and sociobehavioral predictors associated with adherence and retention.

Results: From June 2016 to October 2018, 136 cisgender women enrolled [mean age 40yo (SD 11); 38% non–Hispanic Black and 19% Latina]. In univariate analyses, cisgender Black vs non-Black women (58%, 30%, p=0.003), those attending LA vs San Diego site (79%, 38%, p=0.017) and those having partners of unknown risk vs a partner living with HIV (48%, 25%, p=0.012) were less likely to have consistent TFV-DP ≥4 d/w (findings similar for consistent TFV-DP ≥6 d/w). However, only Black race (OR 0.37, p=0.014) and having partners of unknown risk vs a partner living with HIV (48%, 25%, p=0.012) were less likely to have consistent TFV-DP ≥4 d/w (findings similar for consistent TFV-DP ≥6 d/w). Only pregnancy interest remained significant in multivariable analyses (OR 0.05, p=0.013) was associated with lower likelihood of retention and interest in becoming pregnant in the next 6 months (13%, 32%, p=0.03) with greater likelihood of retention. Only pregnancy interest remained significant in multivariable models (OR 2.81, p=0.042).

Conclusion: In this cohort of cisgender women on PrEP, race and HIV risk group affected adherence whereas severe drug use negatively, and desire to become pregnant positively, impacted retention. Larger prospective studies should evaluate factors associated with long-term adherence and engagement in real-world PrEP settings.

714 WEEKLY ORAL TENOFOVIR ALAFENAMIDE PROTECTS MACAQUES FROM VAGINAL SHIV INFECTION

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Background: Poor adherence to daily pre-exposure prophylaxis (PrEP) reduces efficacy and public health benefit. Simpler oral regimens providing long-lasting protection for one or more weeks may be more desirable among people who have difficulties adhering to a daily pill. Here, we report the pharmacokinetic (PK) assessment of a weekly oral tenofovir alafenamide (TAF) regimen in pigtailed macaques and its efficacy against vaginal SHIV infection.

Methods: We previously defined the human equivalent dose of oral TAF and TDF in macaques to be 1.5 and 22 mg/kg, respectively. Here we assessed in macaques the PK profile of a higher dose of TAF (27.4 mg/kg) in plasma and PBMCs following a single oral dose. Efficacy was determined in macaques that received a weekly 27.4 mg/kg dose of oral TAF and were vaginally exposed to SHIV162p3 at 3- and 6-days post dosing for up to 6 weeks (12 challenges). Infection outcome was compared to 10 untreated macaques. Tenofovir (TFV) and TFV diphosphate (TFV-DP) were measured by LC/MS/MS. SHIV RNA was monitored weekly in plasma by RT-PCR.

Results: Median TFV levels in plasma at 5h were 284 ng/ml (range 227-341). TFV-DP levels in PBMCs (fmo/106/107, cells) were 14,090, 6,740, and 4,390 at 5h, 3 days, and 6 days, respectively. Nine of ten untreated controls were infected after a median of 3 SHIV exposures (range 1-12). In contrast, 5/6 animals receiving a single dose of oral TAF were protected against vaginal SHIV infection (Efficacy = 92.1% [95%CI=39.6%, 99.0%]). Median TFV-DP levels in the protected animals at time of challenge (day 3 and 6) were 6,095 and 3,485 fmo/107, cells, respectively. In contrast, the PrEP breakthrough animal showed much lower TFV-DP in PBMCs at challenges prior to infection (median = 405 [274-677] fmo/107, cells). Using in vivo Cmax, TFV-DP levels and calculated TFV-DP half-life in PBMCs of 5.3 days, we estimate the length of prophylactic window will extend well beyond 1 week.

Conclusion: We identified a dose of oral TAF that resulted in high and sustained TFV-DP levels in PBMCs and protected against vaginal SHIV infection for at least 1 week following a single oral administration. The data open the possibility for long-lasting PrEP protection with infrequent oral dosing.

715 PHARMACOKINETICS OF TAF/EVG RECTAL INSERTS IN MACAQUES AND IMPACT OF RECTAL WASH

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Background: On-demand topical products for HIV prevention may have several advantages, including limited cost and systemic toxicity. CONRAD has developed inserts containing tenofovir alafenamide fumarate (TAF) and elvitegravir (EVG) for on-demand vaginal or rectal pericoital use. We recently showed that TAF/EVG inserts provided high protection as PrEP or PEP in pigtailed macaques exposed vaginally to simian HIV (SHIV). Here, we assessed the PK of the same inserts when applied rectally in pigtailed macaques. Since rectal cleansing is a common practice among men who have sex with men, we also defined the impact of rectal cleansing on drug distribution through the rectum and colon.

Methods: Six female pigtailed macaques received a single insert containing 20 mg of TAF and 16 mg of EVG at 4 cm from the anal margin. Rectal biopsies (8x), rectal secretions, and blood were longitudinally collected at 0, 2, 4, 24, 120, and 168 hours post-application. Drug biodistribution and feces interference were evaluated in 5 additional SHIV positive animals at necropsy. Of these 5 animals, three received rectal washes before dosing. Necropsy was done 4 hours post-dosing, with 8x collection at 4, 8, 15 (rectum) and 25 cm (colon) from the anal margin. Levels of TFV-DP and TAF/EVG in tissues and secretions were measured by LC/MS/MS.

Results: In the longitudinal PK study, TFV-DP levels in rectal tissues peaked at 6 days, and 6 days, respectively. Nine of 10 untreated controls were infected after 3- and 6-days post dosing for up to 6 weeks (12 challenges). Infection outcome was compared to 10 untreated macaques. Tenofovir (TFV) and TFV diphosphate (TFV-DP) were measured by LC/MS/MS. SHIV RNA was monitored weekly in plasma by RT-PCR.

Conclusion: We identified a dose of oral TAF that resulted in high and sustained TFV-DP levels in PBMCs and protected against vaginal SHIV infection for at least 1 week following a single oral administration. The data open the possibility for long-lasting PrEP protection with infrequent oral dosing.
1). Rectal cleansing before insert application increased concentrations of TFV-DP and EVG in the rectum and colon by 40 to 200 times (Table 1).

**Conclusion:** Rectal application of TAF/EVG inserts resulted in tissue EVG and TFV-DP levels at 4 hours that were high and within the range of those associated with vaginal protection. Rectal wash was associated with extended biodistribution of TFV-DP and EVG throughout the rectum and increased tissue drug levels by several orders of magnitude. SHIV challenge studies will help define the rectal protection achieved with TAF/EVG inserts.

### 716 ACCEPTABILITY AND CHOICE FOR 3 PLACEBO PRODUCTS USED WITH RECEPTIVE ANAL SEX


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**Background:** End-user perspectives are vital to the design of new biomedical HIV prevention products. Behaviorally congruent alternatives to condoms and daily oral Pre-Exposure Prophylaxis (PrEP) remain crucial. MTN-035 evaluated the acceptability of and preference for three placebo, non-gel delivery vehicles (insert, enema, suppository) that could be used to deliver PrEP prior to receptive anal sex (RAS).

**Methods:** We enrolled 217 HIV-negative, cisgender men who have sex with men (MSM) and transgender people ages 18–35 into a randomized cross-over trial across 7 sites in the United States, Peru, Malawi, South Africa, and Thailand. Participants were asked to use each product prior to RAS over 4-week periods. At the final study visit, product-experienced participants completed a conjoint experiment where they selected between random sets of product profiles using 7 features (delivery vehicle, timing of use before sex, side-effects, duration of protection, efficacy, frequency of use, and need for a prescription). A subset of participants completed an exit in-depth-interview (IDI; n=70).

**Results:** Participants identified as cisgender men (172; 79.3%), transwomen (42; 19.4%) or transmen (3; 1.3%). Mean age was 24.8 (SD = 4.7 years). Product-experienced participants had heterogeneity in top-ranked product choices across scenarios (Table 1). In conjoint analyses, efficacy was the strongest determinant of stated choice overall (30.4%), followed by product delivery vehicle (18.0%), and side-effects (17.2%). The most common chosen combination of attributes was an enema used — 30 minutes before sex, with 95% efficacy, offering a 3-5 day protection window, used weekly, having no side effects, and available as an over-the-counter product. In IDIs, participants' acceptability across products were informed by the aforementioned features, RAS-related characteristics (e.g., lubricity; hygiene), personal considerations (e.g., relationship status), and social context (e.g., stigma).** Conclusion:** Choice in next generation PrEP products, informed by acceptability and personal preference, is highly desired by MSM and transgender people. MTN-035 participants weighted product features differently, recognizing the potential to create diverse, behaviorally congruent biomedical options that fit the needs of intended end-users. Rather than one-size fits all, our findings underscore the variations in acceptability of non-gel delivery vehicles for local biomedical prevention prior to RAS.

### 717 LONG-ACTING HIV CAPSID INHIBITOR EFFECTIVE AS PrEP IN A SHIV RHESUS MACAQUE MODEL

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**Background:** The ability of daily oral preexposure prophylaxis (PrEP) to effectively reduce the risk of contracting HIV is strongly dependent on high medication adherence, which is not uniformly achieved among people at risk for HIV. Long-acting agents can circumvent the need for daily dosing while providing long-term protection. Lenacapavir (LEN), an investigational small molecule inhibitor of HIV herpetic factor function with picomolar antiviral potency and long-acting subcutaneous (s.c.) dosing potential (twice yearly), is in clinical development for HIV treatment. Here, we evaluated GS-CA1, a LEN analog active against both HIV and SIV, as a long-acting PrEP agent in a macaque rectal SHIV challenge model.

**Methods:** Eight naïve Indian rhesus macaques per group received a single s.c. injection of either vehicle control (placebo), 150 mg/kg (low dose) or 300 mg/kg (high dose) GS-CA1, followed by weekly escalating titer SHIV challenges starting 1-week post-dosing. Blood was collected weekly for the evaluation of plasma drug levels, viral loads, and serology. Animals were considered protected if they remained SHIV-negative by plasma PCR assay and seronegative by enzyme immunoassay throughout the 15-week challenge phase and the 10-week follow-up.

**Results:** Following a single injection, plasma concentrations of GS-CA1 exceeded its serum protein binding adjusted (pa) EC95 value (30.1 nM) for 8-15 weeks and 15-17 weeks in the low and high dose groups, respectively. After a total of 15 challenges 8/8 animals became infected in the placebo group, whereas 2/8 and 5/8 animals remained protected in the low and high GS-CA1 dose groups, respectively. The median time-to-infection was 7.5 weeks in the placebo group, 16 weeks in the low-dose GS-CA1 group, and not reached due to insufficient number of infections in the high-dose group. Relative to the placebo group, the low and high dose treatment groups demonstrated an 86% (p=0.0061) and 96% (p=0.0002) infection risk reduction, respectively, as determined by Cox regression analysis. Notably, based on a 2-week infection-to-detection window, treatment group infections occurred only after plasma GS-CA1 concentrations fell below 2X paEC95.

**Conclusion:** These preclinical data provide a proof of concept for the prophylactic efficacy of a long-acting capsid inhibitor in a nonhuman primate SHIV challenge model and support the clinical development of LEN for HIV prevention.

<table>
<thead>
<tr>
<th>Table 1. Median Drug Concentration in Rectal Biopsies</th>
<th>Most Preferred Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TFV ng/g</strong></td>
<td><strong>TFV-DP (nmol/mg)</strong></td>
</tr>
<tr>
<td>Cleansing</td>
<td>w/o</td>
</tr>
<tr>
<td>Rectum 4 cm</td>
<td>125618</td>
</tr>
<tr>
<td>Rectum 8 cm</td>
<td>46754</td>
</tr>
<tr>
<td>Rectum 12 cm</td>
<td>36753</td>
</tr>
<tr>
<td>Colon</td>
<td>2288</td>
</tr>
</tbody>
</table>

**Suppository (42%)** | **Enema (38%)** | **Insert (20%)** |

**Enema (44%)** | **Insert (29%)** | **Suppository (23%)** |

**Insert (56%)** | **Suppository (31%)** | **Enema (23%)** |

**Insert (59%)** | **Suppository (23%)** | **Enema (10%)** |

Fits your lifestyle if it provided some protection against HIV transmission when used before sex.

**Enema (49%)** | **Insert (29%)** | **Suppository (27%)** |
INFUSION REACTIONS IN THE PHASE 2B ANTIBODY MEDIATED PREVENTION (AMP) STUDIES
Simbarashe Takuvai, Shelly Karuna, Michal Juraska, Erika Rudnicki, Snlatha Edupuganti, Maja Anderson, Robert De La Grecca, Martin R. Gaudinski, Margarita M. Gomez Lorenzo, David Burns, Myron S. Cohen, Lawrence Corey, Kathy Mngadi, Nyaradzo M. Mpofu, for the AMP Study Teams

Methods: Men who have sex with men and transgender individuals at risk for HIV (n=2699) enrolled in Brazil, Peru, Switzerland, and the US (704/085). At-risk, sexually active heterosexual women (n=7924) enrolled in Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, and Zimbabwe (703/081). From April 2016 to April 2020, participants were randomized 1:1 to receive intravenous VIRCO1 at 10 mg/kg, 30 mg/kg, or placebo. In 704/085, 23,867 infusion reactions were administered; in 703/081, 16,807 infusions were administered. Infusions were given every 8 weeks, for 10 infusions total over 72 weeks. Safety was assessed immediately pre- and post-infusion and at monthly follow-up visits.

Results: Forty-seven (1.7%) participants experienced 49 IRRs in 704/085 and 93 (4.8%) experienced 111 IRRs in 703/081 (p<0.01 for a difference by trial). Four IRR clinical phenotypes were observed: urticaria, dyspnea, dyspnea with rash, and "other" reactions including pruritis and flushing. Urticaria was the most prevalent phenotype, occurring in 25 (0.9%) and 41 (2.1%) 704/085 and 703/081 participants, respectively; observed more frequently in VIRCO1 than placebo recipients (92.3% of urticarias in 704/085, 95.1% of urticarias in 703/081); and suggesting a dose-response trend across treatment groups in both trials. In total, 16 participants experienced between two to four IRRs. Most IRRs occurred with the initial infusion (36.7% in 704/085, 30.6% in 703/081). IRRs occurred more frequently in VIRCO1 than placebo recipients in 703/081 (p<0.01), though not in 704/085 (p=0.75). IRRs were associated with atopy in both trials (p=0.01 in 704/085, p<0.01 in 703/081) and with younger age in 703/081 (p<0.01). Of 92 IRRs, six (3.8%) were severe and 96% were mild/moderate. All IRRs were managed successfully without sequelae.

Conclusion: IRRs in the AMP studies were uncommon, typically mild or moderate, successfully managed at the research clinic and fully resolved. Laboratory analysis is ongoing to explore potential mechanisms of these reactions.

Table 1. Number of infusion reactions and participants with an infusion reaction by treatment group and clinical phenotype

1719 INJECTION NETWORKS AND HIV PREVENTION SERVICES AMONG PEOPLE WHO INJECT DRUGS IN INDIA
Neia Prata Menezes, Allison M. McFall, Aylur K. Srikrishnan, Canjeevaram K. Vasudevan, Anand Santhanam, Sunil S. Solomon, David C. Celentano, Gregory M. Lucas, Sheryl H. Mehta
The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, "YR Gaitonde Center for AIDS Research and Education, Chennai, India, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Injection networks of people who inject drugs (PWID) may influence risk behaviors and HIV acquisition. In India, where HIV incidence is rising among PWID, HIV prevention strategies are available, but uptake is poor. We evaluate associations between injection network characteristics and recent engagement in HIV testing in the prior 6 months, medication for opioid use disorder (MOUD) and syringe service utilization in the prior month.

Methods: We recruited 11,745 PWID across 12 Indian cities using respondent-driven sampling in 2016-17. Eligible participants who were ≥18 years old and reported injection drug use in the past 2 years, underwent HIV testing and completed surveys assessing network characteristics, substance use, and HIV prevention program utilization. Among HIV-negative PWID reporting injection in the prior 6 months, we used multilevel logistic regression models, weighted by site-level PWID population size, to evaluate relationships between injection network size and self-reported engagement in each service separately (HIV testing, MOUD, syringe services). Models were adjusted for age, gender, injection frequency, substance use, recent incarceration, and engagement in other services.

Results: 7,380 PWID (63%) were HIV-negative and reported recent injection drug use (median age: 28; 98% male). Recent engagement in HIV prevention services was poor: 12% received an HIV test, 19% engaged in MOUD and 22% in syringe services; 3% engaged in all 3 services. Median injection network size was 3 [IQR: 1-5]; 17% reported injecting with >10 PWID; only 0.9% reported sharing injection equipment with a known HIV-positive PWID in the prior 30 days. Injection network size was not associated with recent HIV testing. Those reporting sharing injection equipment with a known HIV-positive PWID were more likely to report a recent HIV test (adjusted OR [AOR]=2.54; p=0.04). Injecting with >10 vs. 0-1 PWID in the prior 30 days was associated with decreased MOUD use (AOR=0.55; p<0.01) but increased syringe service use (AOR=1.54; p<0.01).

Conclusion: In this large community-based sample across India, injection network size was differentially associated with use of HIV prevention services. These associations may reflect perceived need or access as MOUD and HIV testing tend to be facility based whereas syringe services are commonly community-based. Community-based delivery of HIV testing and MOUD may help overcome barriers.

Table 1. Association between injection drug network characteristics and recent engagement in HIV prevention services among HIV-negative people who inject drugs (PWID) reporting active injection drug use in prior 6 months across 12 cities in India, 2016-17

<table>
<thead>
<tr>
<th>Injection drug network characteristics</th>
<th>HIV testing in the prior 6 months</th>
<th>MOUD use in the prior month</th>
<th>Syringe service program utilization in the prior month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR</td>
<td>p-value</td>
<td>AOR</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-10</td>
<td>0.75</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>&gt;10</td>
<td>0.76</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Shared injection equipment with HIV-positive PWID in prior 30 days a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.05</td>
<td>0.04*</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a All models weighted by estimated site-level PWID population size and adjusted for age, gender, region, household income, marital status, education, incarceration history, frequency of injecting, opioid use, heroin vs. intravenous use, and utilization of two other HIV prevention services.

*Adjusted for site, injection drug quantity, site-level PWID population size, and PWID network member in prior 30 days.

** Statistically significant at α = 0.05 significance level.

MOUD=Medication for opioid use disorder; AOR=Adjusted odds ratio

Notes:
- Participants are counted once within each phenotype. The denominator for percentages is the total number of enrolled participants (N).
- The denominator for percentages is the total number of observed reactions (N)
PREVALENCE & DETERMINANTS OF EARLY SEX RESUMPTION POSTCIRCUMCISION (RAKAI, UGANDA)

Alex Daama1, Doreen Nabakku1, Edward N. Kankaka1, Doreen Tuhebwe1, Absalom Ssettuba1, Fred Naluwoga2, Tom Lutalo3, Joseph Kagaayi4, Gertrude F. Nakigozi2, Michelle Adler1, Lisa Mills1, Ronald Gray3, Maria Waver1, Godfrey Kigozi5

1Rakai Health Sciences Program, Kalisizo, Uganda, 2Makere University, Kampala, Uganda, 3Centers for Disease Control and Prevention, Kampala, Uganda, 4The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Voluntary male medical circumcision (VMMC) reduces HIV infection in men by 50%–60%. The Uganda Ministry of Health (MoH) guidelines recommend abstaining from sexual activity for at least 42 days after VMMC to promote complete wound healing. Early resumption of sexual intercourse post-VMMC increases risk of HIV infection among HIV-negative men and possibly increases risk of male to female transmission. However, previous reports show almost half (48%) of men in Uganda resume sexual activity early following VMMC with higher rates of early sexual resumption (ESR) among married men, men with multiple partners, and men diagnosed as HIV-positive. In view of this, we estimated changes in prevalence and assessed risk factors of ESR among VMMC men in Rakai, Uganda, over an eight-year period.

Methods: We analyzed data from male participants aged 15–49 years in the Rakai Community Cohort Study who self-reported having received VMMC in one of the four successive survey rounds conducted between June 2013 and October 2020 (R1). We estimated ESR prevalence at each round, and to understand risk factors associated with ESR, we used modified Poisson regression to estimate adjusted prevalence ratios (aPR). Statistical significance was tested at 5% level of significance.

Results: Of the 1,832 circumcised men included in the study, 485 (26.5%) reported ESR. ESR significantly decreased over the study period, from 45.1% reported in Round 1 (June 2013 - January 2015); to 31.8% in Round 2 (February 2015 - August 2016); 21.1% in Round 3 (September 2016 - May 2018); and 16.9% reported in Round 1 (June 2013 - January 2015); to 21.8% in Round 2 (February 2015 - August 2016); 17.8% in Round 3 (September 2016 - May 2018); and 16.9% in Round 4 (June 2018 - October 2020).

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BV were described over time; relative risk of correlates of BV were estimated using generalized estimating equations. Models were adjusted for age, sexual activity, income, rural/urban home, STIs, and time from menarche. 

Results: Of 400 AGYW enrolled, 322 (80.5%) reported no prior sexual activity at enrollment. At first visit, 21 of 389 girls (5.6%) had prevalent BV. Over median follow-up of 48 months (inter-quartile range (IQR) 24-57), AGYW had a median of 11 BV tests (IQR = 5-15), 166 (43%) had at least 1 BV diagnosis. Prior to reported sex, 2.8% of visits had BV, compared to 13.7% of visits post sexual activity. BV was 3.5x more likely after sexual debut (95% CI 1.2-3.5, p < 0.001). AGYW reporting more frequent (recent sex (>1 in act in prior 3 months) had higher risk of BV (RR 1.80 compared to those reporting ≤1 act, CI 1.36-2.39; p<0.01). In an adjusted model, sexual initiation remained the most significant risk factor for BV (aRR 2.23, CI 1.13 - 3.9; p<0.01). Other covariates associated with increasing risk of BV included recent sexual activity, urban home, and having no income. STI diagnosis at the same visit was strongly associated with BV, with chlamydia aRR 1.78 (CI 1.4-2.2; p<0.01), gonorrhea aRR 1.77 (CI 1.2-2.4; p<0.01) and increasing risk when BV was reported among those with longer duration between menarche and sex, should be explored.

Risk of bacterial vaginosis among Kenyan adolescent girls and young women, (Adjusted GEE model) 

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Relative Risk (aRR)</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at visit, years</td>
<td>1.00 (0.92, 1.07)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Penile-vaginal sexual intercourse</td>
<td>2.23 (1.28, 3.98)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Penile-vaginal sexual activity in last 3 months</td>
<td>1.38 (1.04, 1.83)</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>Time since last menarche</td>
<td>1.37 (1.07, 1.75)</td>
<td>0.013</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time between menarche and first penile-vaginal sexual intercourse</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5 years</td>
</tr>
<tr>
<td>&lt;5 years</td>
</tr>
</tbody>
</table>

| Chlamydia at same visit as BV                               | 1.78 (1.37, 2.30)   | <0.001                  |
| Gonorrhea at same visit as BV                                | 1.77 (1.06, 2.99)   | 0.034                   |
| Herpes simplex virus 2 antibody positive                    | 1.83 (1.33, 2.50)   | <0.001                  |

### 724 HIV and Syphilis in the District of Columbia

Kaitlin Liloff, 1 Seble Kassaye, 1 Amanda B. Spence, 1 Princy N. Kumar, 2 Madhuri Natarajan, 1 Rachel Harold, 1 Kerri Dorsey, 1 Rupali K. Doshi, 1 Adam J. Visconti 2

1Georgetown University, Washington, DC, USA; 2District of Columbia Department of Health, Washington, DC, USA

Background: HIV and syphilis co-infection comprised 39% of primary or secondary (early) syphilis cases in the District of Columbia (DC) in 2019. These stages of syphilis are associated with increased HIV transmissibility. People living with HIV (PLH) are also at risk for severe manifestations of syphilis. We sought to determine effectiveness of syphilis treatment by assessing time from diagnosis to treatment, variables that influence time to treatment, and adequacy of therapy to identify opportunities to reduce ongoing transmission. Methods: The DC Department of Health created a limited data set of all individuals with syphilis between January 1, 2015 and December 31, 2019. Additional variables included: age, sex, ward, stage of syphilis, diagnosis date, HIV status, CD4+ T cell count, HIV viral load (VL), reporting and treating facility, and treatment type and duration. Adequacy of treatment was delineated by the Centers for Disease Control 2015 syphilis treatment guidelines. Data was analyzed using logistic regression to identify factors associated with delayed treatment >14 days from diagnosis. Results: Among 2,723 individuals, 8% (219/2723) were female, 90.3% (2459/2723) were male, average age was 36.9 years (range: 0 – 93), and 44.7% (1216/2723) were co-infected with HIV. Among 921 PLH with VL data, 39.2% (363/921) had undetectable VL and 26.5% (145/921) had VL > 1,000 copies/mL. Among 893 PLH, 59% (531/893) had a CD4 > 500. Overall treatment adequacy for treatment of early syphilis was 99.9%. Factors associated with delay of treatment included detectable HIV VL (between 20 and 199 copies/mL OR=1.630; p=0.0380; and VL > 100,000 copies/mL OR=1.82; p=0.0387). Other variables associated with delay in treatment are listed in table 1.

Conclusion: Although our dataset demonstrates almost perfect treatment for early syphilis, we identified that nearly a third of PLH co-infected with syphilis have VL > 1000 copies/mL. Unsuppressed VL compounded with significant delays in treatment in this subgroup highlights the importance of targeting public health efforts to engage this population in care. This is necessary.
DISPARITIES IN FAMILY PLANNING AMONG WOMEN LIVING WITH HIV IN THE UNITED STATES

Manasa R. Bhatta1, Alhua Bish1, Jamison Norwood1, Bryan Shepherd1, Jeffrey Nelson1, Imani Ransby1, Megan Turner1, Timothy Sterling1, Jessica L. Castillo1, Vanderbilt University, Nashville, TN, USA

Background: Compared to women without HIV, women living with HIV (WLH) are more likely to use less effective contraception. Unexpected effects of antiretroviral therapy (ART) on pregnancy outcomes have highlighted the need for effective family planning. Contraception use among WLH in the United States over time has not been well described.

Methods: This observational cohort study examined factors associated with contraception initiation among cis-gender women aged 18-45 years in longitudinal care at Vanderbilt’s HIV clinic from 1998-2018. Women with hysterectomies or bilateral tubal ligations (BTL) prior to entry were excluded. Contraception included oral, transdermal, vaginal ring, and injectable hormonal methods; intrauterine devices; and hysterectomies or bilateral tubal ligations (BTL). Annual prevalence estimates of contraception use, weighted for person-time, described contraceptive type, treatment provider type, and effectiveness.

Results: At clinic entry, median age was 31 years (IQR 25-37), median CD4 cell count was 392 cells/μL (IQR 182-444), and 76 (13%) of the 737 women had undetectable HIV viral load. Women were followed for a median of 4.1 years (IQR 1.3-8.1). Only 46 (6%) women were on contraception and 164 (22%) were pregnant at clinic entry. Of the remaining 527 women, 116 (16%) initiated contraception (both p<0.01). Psychiatric comorbidity decreased hazard of contraception and tended to increase hazard of pregnancy. Race, substance use, CD4 cell count, HIV RNA, and ART use were not associated with contraception nor pregnancy.

Conclusion: Most WLH did not use any contraception at baseline nor during follow-up. Pregnancy risk increased with recent clinic entry, whereas contraception initiation remained stable. More effective contraception counseling is needed among WLH, particularly younger women and those with psychiatric comorbidities.

Factors associated with incident contraception use and pregnancy during follow-up

<table>
<thead>
<tr>
<th>Age at clinic entry (ref: 25 years)</th>
<th>Contraception</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of clinic entry (ref: 2008)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV RNA ≤400 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 cell count (ref: 350)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

727 THE PREVALENCE AND CLUSTERING OF MENOPAUSAL SYMPTOMS IN WOMEN LIVING WITH HIV

Hajra Okhia1, Caroline Sabin1, Katharina Haag1, Lorraine Sherr1, Rageshi Dhairyawan1, Richard Gilson1, Burns Fiona1, Shema Tariq1

1University College London, London, UK; 2Barts Health NHS Trust, London, UK

Background: An increasing proportion of women living with HIV (WLWH) are now experiencing the menopause. We describe the clustering of menopausal symptoms in a large, representative sample of WLWH in England to understand the burden and inform care pathways.

Methods: We included 709 women aged 45-60 from PRIME, an observational study of WLWH. The Menopause Rating Scale was used to capture the severity of each of 11 menopausal symptoms (0: None; 1-4: Very severe). Hierarchical agglomerative cluster analysis was used to describe the clustering of symptoms by menopausal status (pre-, peri- and post-).

Results: Median age was 49 years (interquartile range: 47-52). The majority were Black African (71.7%), had completed at least secondary education (89.4%), were currently employed (68.9%) and in a relationship (57.1%). Overall, 211 (29.8%), 117 (16.5%), 182 (25.7%) and 199 (28.1%) women reported no/little, mild, moderate or severe symptoms/complaints, respectively. Joint pain (66.4%) was the most commonly reported symptom, followed by hot flushes (63.0%), exhaustion (61.6%) and sleep problems (61.4%). All symptoms were more common among peri- and post-menopausal women. Amongst pre-menopausal women, joint pain, exhaustion, anxiety, irritability, depression and sleep problems clustered together (Figure). The remaining symptoms (sexual problems, vaginal dryness, bladder problems, heart discomfort and hot flushes) formed a second cluster. Among peri-menopausal women, joint pain clustered more closely with bladder problems, heart discomfort and hot flushes, and there was apparent clustering between vaginal dryness and sexual problems in this group. Among post-menopausal women, the cluster of anxiety, exhaustion, irritability and depression seen in peri-menopausal women remained, with bladder problems and heart discomfort now clustering with vaginal dryness and sexual problems, and joint pain and hot flushes forming a third cluster.

Conclusion: In the first study to explore the clustering of menopausal symptoms among WLWH, we report a high proportion of menopausal symptoms. Whilst exhaustion, anxiety, depression, irritability and sleep problems remained closely related across all menopausal stages, urogenital and somatic symptom clusters become more distinct in the peri- and post-menopause. These data allow a nuanced understanding of symptoms and potential aetiologies in women aging with HIV, facilitating the most appropriate and effective support.
LONG-ACTING COFORMULATED BIODEGRADABLE IMPLANT FOR HIV PREVENTION AND CONTRACEPTION

Linying Li, Archana Krovi, Chasty Norton, Paifio Johnson, Guadalupe Jimenez, Christine Arenes, Ariane Van der Straten, Leah Johnson

1 RTI International, Research Triangle Park, NC, USA, 2 RTI International, Berkeley, CA, USA

Background: Women worldwide face multiple health risks such as unintended pregnancy and HIV. Multipurpose prevention technologies (MPTs) can simultaneously address the need for contraception and prevention of infectious disease with one product. Here, we are developing a long-acting (LA) biodegradable implant comprising a multi-drug formulation for HIV prevention and contraception.

Methods: We selected two well-characterized progestins, levonorgestrel (LNG) and etonogestrel (ENG), and two inhibitors of the reverse transcriptase, the NRTI tenofovir alafenamide (TAF) and an investigational NRTTI. Polycaprolactone extruded tubes were filled with various ARV-hormone combinations and heat sealed. In-vitro release from devices (in phosphate buffered saline, pH 7.4 at 37˚C) was monitored using UV-vis spectroscopy or high-performance liquid chromatography (HPLC), while maintaining sink conditions.

Results: We evaluated 20 MPT multi-drug formulations, each containing varying ratios of an ARV, a hormone, and an excipient (e.g., 50/35/15, 50/25/25 wt.%). Implants comprising the multi-drug formulations exhibit linear release of both ARV and hormone up to 12-months. Variations in the drug to excipient ratios did not affect the release rate, indicating a membrane-controlled release process. Interestingly, the release rates of TAF and ENG are affected by the presence of another drug within the formulations, whereas the release rates of the NRTTI and LNG remained comparable to their respective single-drug formulations (Figure 1). Specifically, co-formulating the NRTTI with ENG lowered the release of ENG while maintaining the release rate of the NRTTI. On the contrary, co-formulation of ENG and TAF exhibited enhanced release rates for both drugs.

Conclusion: We developed a LA MPT implant containing multi-drug formulations for sustained delivery of ARV and hormones with zero-order kinetics. Co-formulating ARVs and hormones showed varied effect in vitro on the release rate of each drug, increasing our ability to tailor the release rates of ARVs and hormones via the co-formulation process. Using a single co-formulated implant can simplify administration and willingness to use and may improve compliance by eliminating the need for insertion of multiple rods containing individual drugs.
Methods: Reports of positive and negative HIV Ab testing from 4 laboratories and a large community testing site (CTS), and HIV VL testing for people living with HIV reported to the San Francisco Department of Public Health were included. We compared the number of HIV Ab and VL tests, and PrEP visits at the CTS each month from January-October 2020 with the corresponding months in 2019. The continuum of HIV care was calculated for new HIV diagnoses in January-June 2020 compared to the same period in 2019.

Results: From January-October 2020, the mean number of monthly laboratory-based HIV Ab tests decreased from 4,400/month in 2019 to 3,644/month in 2020 (Table); and from 1,382/month to 766/month at the CTS. April 2020 had the lowest number of HIV tests, a reduction of 54% in laboratory reporting and 88% in the CTS compared with April 2019; there was a partial rebound through October 2020. While the number of positive HIV tests was lower per month in 2020 compared with 2019, the proportion HIV positive remained stable throughout the study period (2020: Range 0.9-1.4%; 2019: Range 1.1-1.6%). HIV VL testing also declined in 2020 similar to the trend of HIV testing with the largest decline (57%) in April 2020. Overall, PrEP visits at the CTS declined more than 31% in the study period; the largest decline (90%) occurred in April 2020 with partial rebound through October 2020. From January to June 2020, 75 new HIV diagnoses were identified, compared with 101 in 2019. Linkage to care within 1 month was 95% in 2020 and 97% in 2019; HIV viral suppression within 6 months was 75% in 2020 and 76% in 2019.

Conclusion: We have observed substantial reductions in HIV Ab and VL testing during the COVID-19 pandemic, and likely decreased HIV case finding. PrEP care engagement also declined dramatically; however rapid linkage to care and viral suppression after diagnosis remained robust. Continued monitoring of key HIV prevention and care metrics is essential to assessing the complex impact of COVID-19 on the GT2SF goals, and developing tailored mitigation responses.

Table: Year-over-year changes in the number of laboratory-based HIV antibody and viral load tests, and PrEP visits at a large community testing site (CTS) in San Francisco.

<table>
<thead>
<tr>
<th>HIV antibody tests</th>
<th>HIV viral load tests</th>
<th>PrEP visits at CTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>2019</td>
<td>2020</td>
</tr>
<tr>
<td>January</td>
<td>4544</td>
<td>4765</td>
</tr>
<tr>
<td>February</td>
<td>4121</td>
<td>4423</td>
</tr>
<tr>
<td>March</td>
<td>4488</td>
<td>3308</td>
</tr>
<tr>
<td>April</td>
<td>4561</td>
<td>2087</td>
</tr>
<tr>
<td>May</td>
<td>4645</td>
<td>2789</td>
</tr>
<tr>
<td>June</td>
<td>4110</td>
<td>3609</td>
</tr>
<tr>
<td>July</td>
<td>4471</td>
<td>3878</td>
</tr>
<tr>
<td>August</td>
<td>4308</td>
<td>3746</td>
</tr>
<tr>
<td>September</td>
<td>4190</td>
<td>3809</td>
</tr>
<tr>
<td>October</td>
<td>4663</td>
<td>3945</td>
</tr>
</tbody>
</table>

732 IMPACT OF COVID-19 AMONG PEOPLE LIVING WITH HIV IN THE AFRICAN COHORT STUDY (AFRICOS)
Nicole Dear1, Allaahna Esber1, Ajay Parikh1, Emma Duff1, Michael Inozu1, Emmanuel Bahemana1, Hannah Kibuku2, John Owuoth3, Jonath Maswai1, Trevor A. Cowell1, Christina Polyak1, Julie Ake1, U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA, 1Makerere University-Walter Reed Project, Kampala, Uganda

Background: The COVID-19 pandemic and national responses to mitigate this public health threat have disrupted activities of daily living with economic and health impacts globally. The extent of these disruptions is evolving. Our objectives were to characterize participants who missed study visits during the pandemic and assess the impact of COVID-19 on HIV outcomes, employment, food security and trauma among people living with HIV (PLWH).

Methods: AFRICOS began enrolling adults at risk for HIV and PLWH at 12 PEPFAR-supported clinics in Tanzania, Uganda, Kenya, and Nigeria in 2013. At 6-monthly visits sociodemographic questionnaires were administered and clinical outcomes assessed. Chi-squared tests were used to describe differences between those presenting for and missing a study visit since the pandemic began, using data from participants’ most recent visit before 19 March 2020. Generalized estimating equations were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) comparing social and clinical indicators before and during the pandemic. Models were adjusted for age, sex and site; food security models were also adjusted for employment status. Analyses were restricted to those with a pre-COVID-19 visit on or after 1 January 2019 and at least one visit during the pandemic.

Results: As of 1 November 2020, 1023 (44.5%) of 2298 PLWH with a pre-COVID-19 visit since 1 January 2019 had a visit during the pandemic. PLWH who missed a study visit during COVID-19 were less adherent to antiretroviral therapy (ART) (70.0% vs 88.6%, p<0.001), less adherent to clinic visits (77.5% vs 95.4%, p<0.001) and a greater proportion had a viral load ≥1000 copies/mL on ART (17.5% vs 4.6%, p<0.001). Participants seen during the pandemic were less likely to be food secure (aOR: 0.57, 95% CI: 0.44 - 0.74) and more likely to have cut/reduced one or more meals per day (aOR: 1.75, 95% CI: 1.36 – 2.25; table). No significant impacts of COVID-19 were observed on clinic visit or ART therapy (ART) (70.0% vs 88.6%, p<0.001), less adherent to clinic visits (77.5% vs 95.4%, p<0.001) and a greater proportion had a viral load ≥1000 copies/mL on ART (17.5% vs 4.6%, p<0.001). Participants seen during the pandemic were less likely to be food secure (aOR: 0.57, 95% CI: 0.44 - 0.74) and more likely to have cut/reduced one or more meals per day (aOR: 1.75, 95% CI: 1.36 – 2.25; table). No significant impacts of COVID-19 were observed on clinic visit or ART adherence, employment, or Post-Traumatic Stress Disorder. Among 665 PLWH with available data, 597 (88.8%) had a ≥3-month supply of ART during COVID-19.

Conclusion: PLWH missing study visits during COVID-19 were less engaged in care prior to the pandemic. Innovations such as multi-month dispensing, telemedicine, and other differentiated service delivery strategies should be used to retain PLWH in care. During the pandemic, HIV outcomes remained stable, but food insecurity needs to be addressed.
373 POTENTIAL IMPACT OF COVID-19-RELATED DISRUPTIONS ON HIV IN YAOUNDÉ, CAMEROON

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Background: During the COVID-19 pandemic, disruptions to key programs and services may have substantial impacts on populations at risk, including HIV transmission. Here we model the potential impact of disrupted HIV services, especially condom distribution and ART initiation, on HIV incidence among key populations in Yaoundé, Cameroon.

Methods: We used a mathematical model to simulate the potential impact of disruptions to HIV services, including condoms and ART, on HIV incidence among key populations in Yaoundé. The model was calibrated to local demographic, behavioural, and HIV epidemiology data.

Results: A 6-month cessation of ART initiation, condom distribution, and ART services, resulted in an estimated 50% increase in HIV incidence among key populations in Yaoundé, Cameroon.

Conclusion: Disruptions to HIV services and ART may have substantial impacts on key populations in Yaoundé. Ensuring access to ART and condoms is crucial to minimising the impact of the COVID-19 pandemic.

374 VISIT COMPLETION DURING THE TELEMEDICINE TRANSITION IN EARLY MONTHS OF THE PANDEMIC

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Background: Prior to the COVID-19 pandemic, evidence on telemedicine use in people with HIV was limited. In response to the pandemic, telemedicine was widely adopted. We studied the impact of this transition on visit completion.

Methods: We conducted a study of patients in the Johns Hopkins HIV Clinical Cohort. Visit completion was assessed before and after the transition to telemedicine visits. We used logistic regression to determine factors associated with visit completion.

Results: Visit completion increased from 57% to 66% after the transition. Factors associated with higher visit completion included age >60, lower income, and lower clinic appointment frequency.

Conclusion: Telemedicine visits provided access to care, especially for patients with lower appointment frequency. Further study is needed to understand the long-term impact of telemedicine on visit completion.
MODELLING THE IMPACT OF COVID-19-RELATED DISRUPTIONS ON HIV IN THE UNITED STATES

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Background: During the COVID-19 pandemic, gay, bisexual and other men who have sex with men (MSM) in the United States (US) have reported similar or fewer sexual partners and reduced access to HIV testing and care. Pre-exposure prophylaxis (PrEP) use has declined. We estimated the potential impact of COVID-19 on HIV incidence and HIV-related mortality among US MSM.

Methods: We used a calibrated HIV transmission model for MSM in Baltimore, Maryland, and available data on COVID-19-related disruptions from national online surveys of US MSM and from a Boston clinic with extensive PrEP experience to predict impacts of data-driven reductions in sexual partners (0% or 25% - based on different surveys), condom use (5%), HIV testing (20%), viral suppression (VS; 10%), PrEP initiations (72%), PrEP use (9%) and ART initiations (50%), exploring different disruption durations. We estimated the median experience to predict impacts of data-driven reductions in sexual partners (0% or 25% - based on different surveys), condom use (5%), HIV testing (20%), viral suppression (VS; 10%), PrEP initiations (72%), PrEP use (9%) and ART initiations (50%), exploring different disruption durations. We estimated the median

Results: A 6-month 25% reduction in sexual partners among Baltimore MSM, without HIV service changes, could reduce new HIV infections by 12.2% (95%CI: 11.7, 12.8%) and 3.0% (2.6, 3.4%) over 1 and 5 years, respectively. In the absence of changes in sexual behaviour, the 6-month data-driven disruptions to condom use, testing, VS, PrEP initiations, PrEP use and ART use combined were predicted to increase new HIV infections by 10.5% (5.8, 16.5%) over 1 year, and by 3.5% (2.1, 4.5%) over 5 years. A 25% reduction in partnerships offsets the negative impact of these combined service disruptions on new HIV infections (overall reduction 3.9% (-1.0, 7.4%) and 0.0% (-1.4, 0.9%) over 1 and 5 years, respectively), but not on HIV-related deaths (corresponding increases in infections (overall reduction 3.9% (-1.0, 7.4%) and 0.0% (-1.4, 0.9%) over 1 and 5 years, respectively), but not on HIV-related deaths (corresponding increases 6.4% (2.6, 11.9%) and HIV-related deaths by 9.5% (5.2, 15.9%) over 1 year, without changes in sexual behaviour. The predicted impacts of reductions in partnerships or VS doubled if they lasted 12 months or if disruptions were twice as large.
737 COVID-19 PANDEMIC IMPACT ON ACCESS TO HIV SERVICES FOR KEY POPULATIONS IN INDIA
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Background: The COVID-19 pandemic and associated lockdowns threaten to diminish gains made with respect to HIV epidemic control. The impacts are likely to be most profound among marginalized key populations in resource-limited settings.

Methods: Beginning in 2013, we initiated integrated care centers (ICCs) targeting PWID and MSM; ICCs are currently active in 16 Indian cities (8 PWID, 8 MSM) providing core and population-focused HIV services, including HIV counseling/testing, STI testing, and linkage to monitoring of ART from government facilities. To understand the pandemic’s impact on service access, we compared service utilization among ICC clients early in the pandemic (March-July 2020) to pre-pandemic (Jan-Feb 2020) levels. Specifically, we assessed: 1) numbers of clients accessing HIV testing and STI screening as well as new HIV diagnoses, and 2) for HIV-infected clients on ART in December 2019, the medication possession ratio (MPR). The MPR is the percentage of days in a month that a client had an available dose of ART based on the client’s government ART book.

Results: Overall, 14,415 clients visited an ICC from Jan-July 2020. Compared to pre-pandemic levels, the total number of clients receiving services at the ICC began declining in March and dropped to ~25% normal capacity in May and only returned to ~35% capacity by July. HIV testing declined by 88% beginning in mid-March (PWID 90%, MSM 84%) followed by a modest increase in April/May, but levels did not return to pre-pandemic levels (Figure panel A); a similar pattern was seen for STI testing. HIV diagnoses had a sharp decline in March/April with no significant rebound to pre-pandemic levels by July; among MSM there was only one new diagnosis in all of April-July, compared to ~30 each month in January and February. Compared to February, the median MPR in April declined by nearly 60% for PWID (from an MPR of 97% to 40%) and by 20% for MSM (100% to 80%). The MPR continued to fall for PWID reaching a nadir of 16% in July; by contrast the MPR climbed back to near pre-pandemic levels for MSM by July (Figure panel B).

Conclusion: The COVID-19 pandemic has led to significant decreases in use of HIV-related services among key populations in India. PWID have fared substantially worse than MSM in both preventive and treatment services. This presents an opportunity for increased transmission and incidence among groups that are already disproportionately impacted by the HIV epidemic.

738 HIV TREATMENT/RETENTION IN SUB-SAHARAN AFRICA BEFORE AND DURING THE COVID-19 PANDEMIC
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Background: In sub-Saharan Africa (SSA), the COVID-19 pandemic and response has posed a challenge for HIV prevention, testing, and treatment. We used routinely collected President’s Emergency Plan for AIDS Relief (PEPFAR) Monitoring, Evaluation, and Reporting (MER) data to assess potential loss to follow-up (LTU) across PEPFAR countries in SSA before and during the pandemic to determine the impact of COVID-19 on HIV clinical treatment.

Methods: Treatment and LTU data for people living with HIV (PLHIV) aged 15+ from Oct-Dec 2019 (fiscal year[FY]20 quarter[Q]1; pre-COVID) and Apr-Jun 2020 (FY20Q3; during COVID) were extracted from two MER indicators: TX_CURR (PLHIV on treatment) and TX_ML (tracking outcomes of PLHIV potentially LTU) for 18 countries in SSA. Aggregate indicator data are not linked. Data were disaggregated by age band (15–19, 20–29, 30–39, 40–49, 50+), sex, and Proportions of potential LTU were calculated as total number of PLHIV with no known clinical contact since last expected contact divided by total PLHIV on treatment during the reporting quarter. Proportions of select outcomes of potential LTU (Died, LTU <3 months since last expected contact, LTU ≥3 months since last expected clinical contact) were calculated as total individual outcome divided by total LTU. Analyses were disaggregated by reporting quarter, age band, and sex and paired t-tests were run to test for statistical significance between quarters.

Results: Number of PLHIV LTU was 644,380 in FY2021 and 740,112 in FY2023 across the 18 countries. Proportion of any LTU outcome was 4.9% and 5.3% for the two quarters, respectively, and was higher overall among men and those aged 20–29, although not statistically significant (Table). Among all LTU, an increase in deaths and in LTU ≥3 months among men and a decrease in LTU <3 months among women and all age bands were statistically significant.

Conclusion: The proportion of LTU ≥3 months decreased during the early months of the COVID-19 pandemic in SSA, which, in part, may be attributed to adaptations in HIV programming implemented to mitigate further transmission of COVID-19. These data give an initial indication that the COVID-19 pandemic may have implications for HIV treatment in the coming months and ongoing data review is critical.

Table 1. Number and proportion of PLHIV on ART treatment, potential LTU (<3 months), and disaggregated reasons for LTU by country, sex, and age group – 18 months, sub-Saharan Africa, FY2020 and FY2021

<table>
<thead>
<tr>
<th>Country</th>
<th>PLHIV on ART treatment Q1</th>
<th>PLHIV on ART treatment Q3</th>
<th>Potential LTU &lt;3 months Q1</th>
<th>Potential LTU &lt;3 months Q3</th>
<th>Potential LTU ≥3 months Q1</th>
<th>Potential LTU ≥3 months Q3</th>
<th>Sex</th>
<th>Age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>8,155,540</td>
<td>19,914,130</td>
<td>4,601,194</td>
<td>1,977,467</td>
<td>1,486,327</td>
<td>2,430,728</td>
<td>4.9%</td>
<td>15–19</td>
</tr>
<tr>
<td>Mexico</td>
<td>2,863,219</td>
<td>3,630,211</td>
<td>642,135</td>
<td>741,574</td>
<td>546,061</td>
<td>159,727</td>
<td>5.3%</td>
<td>20–29</td>
</tr>
<tr>
<td>South Africa</td>
<td>1,095,084</td>
<td>1,509,894</td>
<td>542,406</td>
<td>681,972</td>
<td>392,513</td>
<td>150,441</td>
<td>5.0%</td>
<td>30–39</td>
</tr>
<tr>
<td>China</td>
<td>4,986,042</td>
<td>6,847,356</td>
<td>2,549,463</td>
<td>3,114,477</td>
<td>1,526,315</td>
<td>694,393</td>
<td>4.6%</td>
<td>40–49</td>
</tr>
<tr>
<td>India</td>
<td>6,481,125</td>
<td>10,157,308</td>
<td>1,227,315</td>
<td>1,683,147</td>
<td>557,664</td>
<td>225,144</td>
<td>5.6%</td>
<td>50+</td>
</tr>
<tr>
<td>Total</td>
<td>32,533,992</td>
<td>39,735,308</td>
<td>11,480,043</td>
<td>13,598,447</td>
<td>4,806,616</td>
<td>2,061,928</td>
<td>5.2%</td>
<td>All</td>
</tr>
</tbody>
</table>

Impact of COVID-19 on Commercial Laboratory Testing for HIV in the United States
Kevin P. Delaney, Praveena Janyathii, Brian Emerson, Weiming Zhu, Marc A. Pittasi, Ya-Lin A. Huang, Kathleen P. Hartnett, Karen W. Hoover
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Background: On March 13, 2020, the United States declared a national emergency to combat coronavirus disease 2019 (COVID-19). Many states and localities issued shelter-in-place or stay-at-home orders to reduce the spread of COVID-19, limiting movement outside the home to essential activities. Since that time the pandemic has been associated with documented disruptions in routine preventive and other nonemergency care. Screening for HIV infection as well as HIV-1 viral load monitoring for persons living with HIV have likely been affected by the pandemic. Laboratory data from the National Syndromic Surveillance Program provide one way to assess the impact of the COVID-19 pandemic on HIV screening, HIV diagnoses and HIV-1 viral load monitoring.
Methods: Using data reported daily to CDC from a large commercial laboratory, we identified lab test reports for HIV screening or HIV-1 viral load testing. For reports with HIV screening test results, we assessed how often the final HIV test algorithm result was confirmed positive. We plotted daily counts of each of the three HIV test types and 7-day moving averages. We also calculated the difference in the number of each type of test performed between March 13, 2019 and September 30, 2019 from those performed during the same period in 2020.

Results: Compared with number of tests performed in 2019, there were 291,047 fewer HIV screening tests, 4,910 fewer confirmed HIV-1 diagnoses, and 67,694 fewer HIV-1 viral load tests performed during March 13 to September 30, 2020. The 7-day average number of HIV tests performed dropped dramatically after March 13, 2020 and did not recover to 2019 levels by September 30, 2020 (Figure).

Conclusion: During the national COVID-19 emergency, routine screening for HIV and HIV-1 viral load monitoring may have been delayed or foregone by many patients and clinicians. Undiagnosed HIV infection and higher viral loads could have led to increased morbidity and transmission. Although the number of tests being performed has partially recovered from a nadir this spring, testing at this commercial lab has not yet rebounded to make up what was lost. Healthcare system adaptations including home testing, home sample collection, and telemedicine visits for HIV care can help to address this shortfall as the COVID-19 pandemic persists in the US.

740 PROJECT CoRECT (COOPERATIVE RE-ENGAGEMENT CLINICAL TRIAL): FINAL CONNECTICUT RESULTS
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Background: The CDC sponsored Cooperative Re-Engagement Controlled Trial (CoRECT) tested a Data to Care (D2C) strategy that was based on a collaborative approach between health departments and HIV clinics to identify, re-engage, retain and virally suppress persons with HIV (PWH) who were recently out-of-care (DOC). CT was one of 3 sites that participated in this first randomized control trial (RCT) to test efficacy of this approach.

Methods: The CT DPH, Yale University School of Medicine and 23 HIV clinics conducted the study. Using the DPH eHARS surveillance database and individual clinic level data, PWH defined as DOC (lack of HIV VL and/or clinic visits 6 months after a 1 year in-care period) were investigated by clinic personnel to assess eligibility for randomization to clinic standard of care (SOC) vs DPH field epidemiologists (DIS or disease intervention specialists) who were trained to locate, assess barriers to care, and facilitate re-linkage to care. Primary outcomes were: re-engagement at 90 days, retention in HIV care at 12 months, viral suppression (VS) at 12- and 18-months post randomization.

Results: There were 655 patients randomized: DIS (N=333) vs. SOC (N=322), mean age was 46.1 years; 62.4% were male; 37% were Hispanic, 40.3% were black, 20.8% white; 25.6% MSM, 27.2% IDU, 29% heterosexual; there was no significant difference between the 2 arms. For primary outcomes: re-engagement at 90 days, DIS 170 (51.1%) vs SOC 135 (41.9%), p=0.019; retention in care at 12 months, DIS 167 (50.2%) vs SOC 157 (48.8%), p=0.72; VS at 12 months, DIS 221 (74.8%) vs SOC 187 (70.8%), p=0.56; VS at 18 months, DIS 221 (66.4%) vs SOC 205 (63.7%), p=0.47. PWH who were re-engaged in care at 90 days (either DIS or SOC) were more likely to be retained at 12 months, p<0.001.

Conclusion: 1) A Data to Care process involving active input from HIV clinics in collaboration with CT DPH successfully identified recently DOC clients by a joint data sharing and case evaluation process 2) The DIS intervention was successful in re-engaging DOC PWH at 90 days but not in longer term outcomes such as retention in care at 12 months and VS at 12 and 18 months. 3) Re-engagement in care at 90 days was associated with increased likelihood of retention at 12 months 4) For this recently DOC group, these remain gaps in all primary outcomes, suggesting that additional interventions are needed 5) The D2C approach created a working relationship between DPH and HIV clinics which is key to improvements in local HIV care cascades.
2020. Patients received a Bluetooth-enabled pulse oximeter and smartphone application (Patient-M-Power®) and uploaded twice-daily SpO2 readings, heart rate and dyspnoea score (1-10). A team of 24 nurses confirmed results. Absent or absent data triggered calls from the CVC, with assessments and/or admission as required. We collected data on demographics, calls received from home, patients, outcomes and readmissions. Descriptive analysis of the CVC was performed as well as logistic regression to explore factors associated with the likelihood of readmission.

**Results:** 502 patients were included (179 [36.4%] male, median age 39 (IQR 50-3) years, 360 (73.2%) staff. Outcomes are illustrated in Figure 1. Median time in CVC was 12 days (IQR 13-10). 192 calls were made to patients by CVC staff prompted by abnormal data: dyspnoea (41 patients, 8.2%), low SpO2 (133, 26.5%), tachycardia, (99, 19.7%), technical issues (81, 16.1%), absent results (255, 50.1%). This resulted in 45 (9%) patients requiring re-assessment and 42 (8.4%) being readmitted. Of those readmitted, 3 (7%) required critical care admission. Median length of stay was 2 (IQR 6.75-1) days. Those readmitted were more likely to be older (odds ratio [OR] per year older 1.03 (1.01, 1.05), P=0.005), have an abnormal SpO2 (<94%, OR 5.43 (2.93, 9.3), P<0.001), a high dyspnoea score (>7, OR 4.33 (2.04, 9.3), P<0.001) and be staff (OR 6.08 (3.11, 11.87), P<0.001). Neither gender nor abnormal HR were associated with higher likelihood of readmission. 22.2% of presenting patients were hypoxic in the absence of dyspnoea, of which 70% required admission and one patient required intensive care.

**Conclusion:** We describe the largest remotely monitored cohort of COVID-19 patients to date. The low frequency of readmissions and value of SpO2 monitoring and dyspnoea scores as predictors of readmission highlights the value of this model in providing safe care whilst minimising unnecessary admissions.

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**Table 1.** Characteristics of participants and key outcomes of telephone survey completed by women living with HIV about changes in access to HIV and family planning (FP) care during the COVID-19 pandemic (N=784).

<table>
<thead>
<tr>
<th>Characteristic/Outcome</th>
<th>N (%) or median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>37 (29-48)</td>
</tr>
<tr>
<td>Married or cohabitating (n=774)</td>
<td>436 (56)</td>
</tr>
<tr>
<td>Number of living children</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>Attend a HIV clinic at MTRH*</td>
<td>273 (35)</td>
</tr>
<tr>
<td>Time on ART, years</td>
<td>9 (4-12)</td>
</tr>
<tr>
<td>Use of family planning* (n=783)</td>
<td>549 (69)</td>
</tr>
<tr>
<td>Doulegravir as ART basel</td>
<td>529 (65)</td>
</tr>
<tr>
<td>Difficulty refilling medications</td>
<td>249 (32)</td>
</tr>
<tr>
<td>Difficulty obtaining medical care</td>
<td>107 (14)</td>
</tr>
</tbody>
</table>

* = number of participants

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**743 CHANGES IN ACCESS TO CARE FOR WOMEN LIVING WITH HIV DURING THE COVID-19 PANDEMIC**

Caitlin Bernard1, John M. Humphrey2, Julie Thorne1, Shukri Hassan1, Victor Omordi, Beatrice Jakai1, Kara Wools-Kaloustian1, Rena Patel2, Mercy Maina1

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**Background:** The COVID-19 pandemic has disrupted health services globally. We examined the self-reported impact of the pandemic on access to HIV and family planning (FP) services among women living with HIV (WLHIV) at a large HIV treatment program in Kenya.

**Methods:** Telephone surveys were conducted among a non-random, purposeful sample of WLHIV ≥15 years of age exposed to dolutegravir (DTG) at HIV clinics affiliated with the Academic Model Providing Access to Healthcare (AMPATH), a PEPFAR-supported treatment program in western Kenya. Participants completing phone interviews for a DTG-focused study named Chaguo Langu were asked structured questions about their HIV and FP experiences and pregnancy intentions in the context of the pandemic. Multivariable logistic models were used to estimate the odds of self-reported difficulty refilling medications and obtaining care, respectively, adjusted for age, partner status, number of children, time on ART, and HIV clinic site.

**Results:** Among 814 women called from June-October 2020, 784 (96%) completed the survey. Overall, 14% reported experiencing increased difficulty obtaining care during the pandemic, primarily due to cost or unavailability of transportation (61%). Further, 32% reported increased difficulty refilling medications during the pandemic, primarily due to medication stock-outs (79%). However, only 2% reported missing medication doses. Only 2% reported increased difficulty managing (including initiating, refilling, and removing) FP methods, primarily due to stock-outs of implants and injectables (69%). Most (95%) reported no impact of the pandemic on their intentions to use FP or become pregnant. None of the factors assessed in the multivariable model were associated with difficulty obtaining care. Older women were less likely (aOR=0.95, 95% CI: 0.92-0.98), and women with a higher number of children were more likely (aOR=1.13, 95% CI: 1.00-1.28) to have an association with difficulty refilling medications.

**Conclusion:** A significant proportion of WLHIV report experiencing greater difficulty obtaining HIV care and medication refills during the pandemic, while women’s ability to manage FP during the pandemic largely remained stable. Addressing medication stock-outs and transportation challenges may help HIV programs in resource-constrained settings ensure that access to HIV and FP services is not disrupted during the COVID-19 pandemic. As the pandemic continues, FP access and use should continue to be monitored to avoid unintended pregnancies.

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**744 BARRIERS IMPACTING TELEHEALTH MEDICAL-APPOINTMENT ADHERENCE AMONG PLWHA**

Nicole Ennis1, Laura Armas2, Seyram Butame3

1Florida State University, Tallahassee, FL, USA, 2C4N Community Health, Sarasota, FL, USA

**Background:** Access to medical treatment and care for those living with or at risk for HIV is vital to ensuring quality of life and limiting the spread of the disease. Lack of access to medical care is associated with poor disease management, antiretroviral medication failure, and increased incidence of ER visits and/or hospitalizations. To address the medical care needs of patients living with HIV, providers have adapted to telehealth protocols that have allowed them to examine, assess and treat patients using secure 2-way video platforms with audio capabilities. While telehealth has been successfully implemented for patients in routine care, lack of access due to the digital divide has not been closely examined. The goal of the current study was to characterize the response of patients to telehealth during the first six months of active telehealth care.

**Methods:** Due to COVID-19 pandemic, CAN community clinics transitioned to video telehealth visits. Appointment adherence data for this study was taken from the electronic health record appointment using April 1, 2020-October 31, 2020 timeframe. No shows, cancellations and rescheduled appointments were excluded.

**Results:** We identified 5,470 unique patients who completed a total of 12,345 visits at CAN community locations, 80% of clinics were located in Florida. The average age of the population 48.62 yrs with SD of 13.37 yrs with range of 16 yrs - 88 yrs. 88% of patients had a confirmed HIV diagnosis and 12% of patients were on PrEP, majority (78%) identified as male, 62% identified as MSM, and 60% identified as White. Descriptive analysis shows that Blacks were 15% and those
COVID-19 IMPACT ON THE COST OF INDEX TESTING HIV CASE DETECTION IN 5 INDIAN DISTRICTS

Salin Sridomporn, Rose Pollard, Gincy Thomas, Aylur Kailasam Ganesh, Ajay K. Enugu, Subash Gosh, Aditya Singla, Jalak Thakker, Sunil S. Solomon, Bryan Patenaude

The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, and the Johns Hopkins University School of Medicine, Baltimore, MD, USA. 1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, and the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

Background: Index testing is a viable strategy to identify HIV cases globally. However, the impact of the COVID-19 pandemic on the cost of index testing in resource-constrained settings has not been studied in depth.

Methods: Program ACCELERATE implemented Facility-Based Index Testing (FBIT; n=5 districts) and Community-Based Index Testing (CBIT; n=3 districts) in 2 high-burden Indian states. Retrospective costing data were obtained from expenditure records, including cost of labor, supplies/equipment, capital, training, and operational costs. Fixed and recurrent costs at the programmatic level, both overall and per district, were estimated, excluding the cost of HIV testing. On 03/24/2020, India implemented a nationwide lockdown. 10/2019-03/2020 was classified as pre-COVID and 04/2020-09/2020 as post-COVID. To address the digital divide, 100 respondents were asked to verify their identity and provide consent. Staff used a structured questionnaire to collect self-reported data on demographic characteristics, experience of COVID-19 symptoms (CS) within the past 14 days as defined by the World Health Organization, access to health services, and ART interruptions (≥1 dose missed in past week) during the pandemic. We summarized data using proportions and medians and used Chi-square tests to examine associations.

Results: From August-October 2020, we dialed 17,944 numbers; 26.1% (4,680) confirmed their identity, and 98.6% (4,385) eligible clients consented, and 98.6% (4,385) of all respondents, 17.6% reported not accessing health care services during the pandemic. Challenges included health facility closures (13.6%), no money for transport (13.9%) and fear of COVID-19 (45%). Few respondents (1.8%) reported missing ART doses.

Discussion: Declines in PLHIV ART access and worsened mental health may have contributed to increased rates of COVID-19 symptoms. Innovative strategies, such as integrating home-based testing and HIV self-testing, may be required to offset travel restrictions imposed by COVID-19 and improve program efficiency, while minimizing exposure to SARS-CoV-2.

Table 1: Syndrome surveillance for COVID-19 and health care access among ART patients, Malawi

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Pre-COVID</th>
<th>Post-COVID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent cough</td>
<td>27% (n=98)</td>
<td>35.7% (n=101)</td>
</tr>
<tr>
<td>Headache</td>
<td>18.6% (n=62)</td>
<td>18.6% (n=62)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>11.9% (n=42)</td>
<td>11.9% (n=42)</td>
</tr>
<tr>
<td>Runny nose</td>
<td>11.1% (n=37)</td>
<td>11.1% (n=37)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>10.3% (n=34)</td>
<td>10.3% (n=34)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>9.7% (n=33)</td>
<td>9.7% (n=33)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6.1% (n=21)</td>
<td>6.1% (n=21)</td>
</tr>
<tr>
<td>Asthma symptoms</td>
<td>3.9% (n=13)</td>
<td>3.9% (n=13)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3.3% (n=11)</td>
<td>3.3% (n=11)</td>
</tr>
<tr>
<td>New symptoms</td>
<td>1.3% (n=4)</td>
<td>1.3% (n=4)</td>
</tr>
</tbody>
</table>

476 SYNDROMIC SURVEILLANCE FOR COVID-19 AND HEALTH CARE ACCESS AMONG ART PATIENTS, MALAWI

Thulani Maphosa, Thoko Ch. Kaluza, Brittney N. Baack, Evelyn Kim, Joram L. Sunguti, Anne Chauma-Mwale, Rhoderick Machekano, Alice N. Makula, Andrew S. Azman, Andrew F. Auld, Suzgo Zimba, Harri Nikkola, Rachel Kanyenda, Rose Nyirenda, Godfrey Woelk, Elizabeth Genser Pediatric AIDS Foundation, Lilongwe, Malawi, Department of HIV and AIDS, Lilongwe, Malawi, and Center for Disease Control and Prevention, Lilongwe, Malawi. 1Public Health Institute Malawi, Lilongwe, Malawi, 2Elizabeth Genser Pediatric AIDS Foundation, Washington, DC, USA, and Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

Background: While little is known about the interaction of HIV and SARS-CoV-2, disruptions caused by the COVID-19 pandemic may impact the ability of people living with HIV (PLHIV) to access antiretroviral therapy (ART). We conducted syndromic surveillance to identify challenges in PLHIV's access to health care services in selected districts in Malawi.

Methods: We conducted telephone-based syndromic surveillance among randomly selected PLHIV ≥18 years old who had a telephone number on file in 179 ART clinics across nine districts. Patients who answered the phone were asked to verify their identity and provide consent. Staff used a structured questionnaire to collect self-reported data on demographic characteristics, experience of COVID-19 symptoms (CS) within the past 14 days as defined by the World Health Organization, access to health services, and ART interruptions (≥1 dose missed in past week) during the pandemic. We summarized data using proportions and medians and used Chi-square tests to examine associations.

Results: From August-October 2020, we dialed 17,944 numbers; 26.1% (4,680) confirmed their identity, and 98.6% (4,385) eligible clients consented, and 98.6% (4,385) of all respondents, 17.6% reported not accessing health care services during the pandemic. Challenges included health facility closures (13.6%), no money for transport (13.9%) and fear of COVID-19 (45%). Few respondents (1.8%) reported missing ART doses.

Discussion: Declines in PLHIV ART access and worsened mental health may have contributed to increased rates of COVID-19 symptoms. Innovative strategies, such as integrating home-based testing and HIV self-testing, may be required to offset travel restrictions imposed by COVID-19 and improve program efficiency, while minimizing exposure to SARS-CoV-2.
747  **UPSCALING HIV PREEXPOSURE PROPHYLAXIS IMPLEMENTATION DURING COVID-19 PANDEMIC**

Tamirirashë C. Mahwëre1, Nthabiseng Koloane1, Jacqueline Burgess1, Ziyanda Makaba1, Claire Serra1, Todd Malone1, for the Provincial Departments of Health Mpumalanga KwaZulu-Natal South Africa

**Broadreach Corporation, Cape Town, South Africa**

**Background:** HIV Pre-Exposure Prophylaxis (PrEP) was introduced in South Africa in October 2019. Low levels of PrEP uptake and retention were observed through May 2020. The Coronavirus Disease 2019 (COVID-19) pandemic and nationwide lockdown in March 2020 contributed to additional challenges in PrEP uptake in the following ways: • Reduction in HIV Testing Services (HTS) due to facility headcount reduction and suspension of community HTS • Healthcare facility closures and/or reduction in staff after confirmed COVID-19 cases. • Suspension of in-person trainings on PrEP scheduled for March-May before all clinicians were trained – Reduction in onsite mentorship

**Methods:** After a decrease during lockdown in April, we conducted first round of virtual trainings in May Over June-August 2020, we implemented a PrEP acceleration plan that included the following strategies: • Provision of performance targets with online coaching and mentoring for clinical staff • Virtual guidelines training of 300 clinical staff • Printing and distributing IEC materials and job aids • Integration of PrEP into HTS including HIV Self-Screening and Index testing • Stringent monitoring of PrEP drug stock and performance • NDHO-endorsed multi-month dispensing of PrEP drugs

**Results:** During lockdown, PrEP initiations decreased by 40% between March and April and following the first round of virtual trainings, increased by 182% between May and June 2020. The growth plateaued in July, before implementation of the PrEP acceleration plan which catalysed a significant growth spurt both in August of 110% (1,413/764) and in September 102% (2,753/1,413) with these two months alone accounting for 67.4% (1,857/2,753) of the total clients initiated since the inception of the programme.

**Conclusion:** A multi-pronged approach to manage the challenges caused by the COVID-19 pandemic succeeded in improving PrEP initiation and retention. We recommend sustained medicine availability, virtual trainings and mentorship sessions combined with PrEP/HTS integration be implemented to improve upsampling of PrEP services during a pandemic and nationwide lockdown.

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748  **IMPACT OF LOCKDOWN RESTRICTIONS DUE TO COVID-19 PANDEMIC ON HIV CARE IN ITALY**

Andrea Antinori1, Alessandro Tavelli1, Cristina Musinì1, Andrea Gori1, Franco Maggiolo1, Antonella Castagna2, Francesca Ceccherini-Silberstein1, Sergio Lo Caputo1, Massimo Puoti1, Carmela Pinet1, Valeria Calvino1, Enrico Girardi1, Carlo F. Perno1, Antonella Adrigno Monforte1,2, Alessandro Cozzi-Lepri1,2

1Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy; 2Icona Foundation, Milan, Italy; University of Modena and Reggio Emilia, Modena, Italy; Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; Azienda Ospedaliera Pugliese Giacomo XXIII, Bergamo, Italy; San Raffaele Vita-Salute University, Milan, Italy; University of Rome Tor Vergata, Rome, Italy; University of Foggia, Foggia, Italy; ASET Grande Ospedale Metropolitano Niguarda, Milan, Italy; 3AILAIDS ONLUS, Rome, Italy; 4Bambino Gesù Children’s Hospital, Rome, Italy; 5Azienda Ospedaliera San Paolo, Milan, Italy; 6Modelling and Evaluation (CREME) Institute for Global Health UCL, London, UK

**Background:** It has been observed that lockdown restrictions during COVID-19 pandemic may have had a negative impact on HIV epidemic goals with disruption in care. We aim to analyse the trends in non-viral suppression for PLWH during and after the lockdown for COVID-19 pandemic in Italy compared to 2019.

**Methods:** We included all participants in the ICONA cohort for whom there was ≥1 viral load (VL) in the window Nov 2019-Jan 2020 and with most recent VL≤50 copies/mL (exposed to lockdown), and over Nov 2018-Jan 2019 (not exposed). New enrolments in the study period were excluded. At population level and separately by year, we calculated proportion with VL≤50 copies/mL at each month over March-September and we performed an intermittent time series (ARIMA) model centred in March. In addition, we defined an individual outcome using the first VL over May-September (>50 vs. ≤50 copies/mL), comparing proportion with VL>50 copies/mL between exposed and not exposed by means of logistic regression models. PLWH with missing VL in the outcome window were excluded from the analysis. We also performed an alternative analysis in which censoring bias was minimised using inverse probability of weighting. Sensitivity analyses were performed after restricting to clinical sites with electronic linkage with laboratory data and to the subset of PLWH under follow-up in both years.

**Results:** A total of 3,684 PLWH were included (2019=2,948; 2020=736). PLWH exposed to lockdown were significantly older, less frequently MSM, non-Italian, had a higher CD4+ count and more frequently resident in north of Italy. The mean proportion of VL≤50 copies/mL was 97% at March 2020 (ref.), 99% before March 2020, 82% at April 2020 (ARIMA estimates -2% 95% CI:-28%-14%; P=0.01) and 97% after April 2020. In the 2019, the same proportions were 100%, 98%, 95%, and 97% with evidence for a lower drop in April (-6%, 95% CI:-8%–3%; p=0.02). The results of the logistic regression model are presented in Table 1. When restricting to sites with electronic VL linkage and to those followed-up in both years the IPW OR of 2020 vs. 2019 were 1.23 (0.69-2.18) and 1.03 (0.49-2.19), respectively.

**Conclusion:** We found little evidence for a difference in the proportion of PLWH with a VL>50 copies/mL, following stable suppression, in the period post lockdown due to COVID-19 as compared to the previous year. Although selection bias was minimized, reasons for a missing VL should be further investigated.

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749  **INTERVENTIONS TO IMPROVE HEADCOUNT DURING COVID-19 LOCKDOWN IN SOUTH AFRICA**

Dhrisra Naidoo1, Nthabiseng Koloane1, Claire Serra1, Taryn Arthurs1, Ntombifikile Thekiso1, Calvin Moyana1, Todd Malone1, for the Department of Health

**Broadreach Corporation, Cape Town, South Africa**

**Background:** The world was overcome by the COVID-19 pandemic from late 2019. South Africa entered into a country wide lockdown level 5 from March 26 to April 16, 2020. Public health facilities were greatly affected as they experienced reduced facility headcounts, which resulted in reduced HIV testing services (HTS), reduced patients attending their follow-up visits, and this also impacted the viral load completions.

**Methods:** This was a retrospective review that analyzed the trends and the impact COVID-19 had on the headcount of primary health care (PHC) facilities and the number of patients accessing HTS and the Total Remaining on ART (TROA). In order to view the facility headcount and HTS trends on the same scale, we defined an individual outcome using the first VL over May-September (>50 vs. ≤50 copies/mL), comparing proportion with VL>50 copies/mL between exposed and not exposed by means of logistic regression models. PLWH with missing VL in the outcome window were excluded from the analysis. We also performed an alternative analysis in which censoring bias was minimised using inverse probability of weighting. Sensitivity analyses were performed after restricting to clinical sites with electronic linkage with laboratory data and to the subset of PLWH under follow-up in both years.

**Results:** Facility headcount dropped during the COVID-19 period (Mar 20, 0.98 to Apr 20, 0.73), and it is clear that the HTS trends (Mar 20, 0.89 to Apr 20, 0.73) were similar.
0.47) mirror the headcount trends (Figure 1). However, the total remaining on ART remained relatively stable during this period; demonstrating successful programme efforts towards retention. These activities included case management of clients, community ART delivery, SMS reminders, extension of CCMDD (Centralised Chronic Medication Dispensing and Distribution) scripts, multi-month scripting and dispensing, improved appointment systems in facilities where we had interns, data quality improvement activities during this period and daily tracking with the district teams. Historically we have seen that HTS habitually drops during the holiday periods of December and April but starts picking up and follows the headcount trends. This year Level-5 lockdown brought on a steep drop with a strong recovery once lockdown restrictions eased, albeit not totally to former levels.

Conclusion: Therefore, despite drastic drops in headcount from April 2020 to September 2020 as compared to 2019, the stability of TROA shows that implemented retention strategies have had a positive impact on the retention of patients during a pandemic.

750 COMPARISON OF COMMUNITY TESTING OUTCOMES OVER SOUTH AFRICA COVID-19 LOCKDOWN LEVELS

Jacqueline Burgess1, Stephanie Berrada1, Hilton Julius1, Claire Severo1, Dhirisha Naidoo1, Nthabiseng Kolone2, Shuahe Rajap1, Todd Malone1, for the Mpumalanga and KwaZulu-Natal Provincial Research Group

1BroadReach Corporation, Cape Town, South Africa, 2Hospice Palliative Care Association of South Africa, Pretoria, South Africa, ‘CareWorks, Cape Town, South Africa

Background: BroadReach Healthcare is a PEPFAR district support partner in four districts in two South African Provinces: Mpumalanga (MP) and KwaZulu Natal (KZN). CareWorks and Hospice Palliative Care Association of South Africa (HPCA) are organizations implementing community-based HIV testing services (HTS) under BroadReach Healthcare. During the COVID-19 level-5 lockdown time period, community-based HTS was halted and confined to areas immediately outside of healthcare facilities, and in MP, community-based Lay Counsellors provided HTS in facilities. The aim of this evaluation is to compare HTS rates observed during three 5-week time intervals in 2020: pre-level-5 lockdown (19 February–25 March), during level-5 lockdown (26 March–30 April), and after level-5 lockdown (01 May–06 June).

Methods: We conducted an operational evaluation of community and facility data from CareWorks and HPCA: HTS completed, HTS with positive results (HTS_TST_POS), and testing yield. Chi-square tests were used to determine statistical significance.

Results: Testing decreased in all districts, averaging a 43.1% drop (4,809/11,166) from pre-level-5 lockdown to level-5 lockdown, then rebounded to 91.4% of baseline (10,208/11,166) afterwards (p < 0.05). Over the same timescale, HTS_TST_POS decreased by only 6.3% (419 from 447) before reverting to 97.1% (407/424) of its pre-level-5 lockdown performance (p < 0.05). This was inconsistent across provinces: case finding increased in MP by 64.6% (321 from 195), despite decreased testing, and decreased in KZN by 61.1% (98 from 252). Testing yield was highest during level-5 lockdown in both provinces; averaging 8.7% (419/4,809) compared to 4.0% before (447/11,166) and 4.0% after (407/10,208) level-5 lockdown (p < 0.05). MP’s testing yield increases far exceeded observed increases in KZN between pre-level-5 lockdown and level-5 lockdown time intervals: 9.2% from 3.0% in MP and 7.4% from 5.4% in KZN (p < 0.05).

Conclusion: The allocation of community-based Lay Counsellors inside and outside of healthcare facilities during the South African COVID-19 level-5 lockdown assisted with finding people who are HIV-positive, and who are presumably at higher risk of developing severe COVID-19 disease.

751 A SURVEY-BASED PILOT STUDY TO ASSESS THE EFFECTS OF COVID-19 ISOLATION ON OLDER PLWH

Lauren Smith1, Hannah Walsh1, Jennifer Chiarella1, Julian Weiss1, Serena S. Spudich1, Shelli F. Farhadian2

1Yale University, New Haven, CT USA

Background: Public health emergencies increase stress, anxiety, and fear, and older adults and those with pre-existing conditions may be especially vulnerable. We used a survey-based pilot study to explore the psychosocial impact of COVID-19 on older PLWH and correlate the level of COVID-19 related distress with baseline HIV disease metrics.

Methods: Participants were PLWH > age 50 who had previously (2017-2020) enrolled in the HARC HIV biorepository study at Yale. 48 PLWH were contacted and 22 participated in this study, conducted Aug-Sep 2020. An 8-part survey was administered to inquire about COVID-19 exposure, financial distress, medication adherence/medical follow-up, social support, substance use, and mood symptoms (Table 1). Cross-sectional analysis was performed on results at the time of survey administration, and longitudinal analysis was performed to compare anxiety (GAD-7), alcohol/drug use (ASSIST), and depression (CES-D) to baseline values obtained pre-pandemic (median 1.3 years prior).

Results: Participant demographics are reported in Table 1. 2 participants reported having been diagnosed with COVID-19, 1 of whom had a known COVID-19 positive contact. 68% of participants were retired and reported no changes to their work due to COVID-19, and most reported moderate (4.1 on scale of 0-7) financial distress. Most reported excellent medication adherence, with 77% reporting no missed doses. 95% stated they felt “very well supported” by their primary HIV care providers, with 18% saying their care was improved during COVID-19. Only 18% felt their care was “somewhat worse.” Most participants also scored highly on the social support scale, with an average score of 11 out of 14. There were no significant differences between pre-pandemic and current scores for anxiety, alcohol/drug use, and depression, and there was no correlation between baseline HIV metrics and current level of distress. However, there was an association between COVID-19-associated worsening in GAD-7 score and a history of substance use disorder (p = 0.02).

Conclusion: These results suggest that overall, most participants were doing well with excellent medication adherence and no significant changes in scores for anxiety, depression, and substance use, but that older PLWH with a history of substance use disorder had a greater risk for increased anxiety during COVID-19. These findings can help identify groups who may be the most at-risk to experience distress from a second wave of COVID-19 and put support measures in place.
HIV AMBULATORY CARE DURING COVID-19 PANDEMIC IN US: VISITS AND VIRAL LOAD TESTING

Ellen M. Tedaldi1, Qi Jiang Hau1, Carl Armon3, Frank Palella3, Jun Li4, Gina Simoncini5, Jack Fuhrer5, Cynthia Mayer6, Kimberly J. Carlson6, Kalliope Chagaris7, Kate Buchacz1, Simoncini8, Ellen M. Tedaldi1, Carl Armon3, Frank Palella3, Jun Li4, Gina Simoncini5, Jack Fuhrer5, Cynthia Mayer6, Kimberly J. Carlson6, Kalliope Chagaris7, Kate Buchacz1

1Temple University, Philadelphia, PA, USA, 2Cerner Corporation, Kansas City, MO, USA, 3Northwestern University, Chicago, IL, USA, 4Stony Brook University, Stony Brook, NY, USA, 5St Joseph’s Comprehensive Care Institute, Tampa, FL, USA

Background: COVID-19 pandemic effects on ambulatory care services for persons living with HIV in the United States, including on frequency of office visits and HIV viral load (VL) testing, have not been well described.

Methods: We analyzed longitudinal data of active patients (any encounter after January 1, 2020) at 8 HIV Outpatient Study (HOPS) sites. Monthly rates of all-inclusive encounters (office, lab, pharmacy, hospital, telemedicine [TM], phone, other), office and TM (O&T) visits, and HIV VL tests were derived from generalized linear mixed models, using data available from January 2010 to June 2020. We then assessed temporal trends and the adjusted effects of sociodemographic and clinical factors on the rates of O&T visits during the COVID-19 pandemic, in multivariable Poisson regression of data from January to June 2020.

Results: Of 1251 active patients, 71% were male, 57% aged ≥ 50 years, 36% non-Hispanic white, 42% non-Hispanic black, 19% Hispanic/Latino, and 49% publicly insured. Median CD4 count was 680 cells/mm, and 93% had suppressed (<200 copies/mL) VL on last test before January 1, 2020. Patients contributed 10,041 person-years of observation from January 2010 to June 2020. Monthly all-inclusive visit rate (95% Confidence Interval) dropped from 0.33 (0.31, 0.34) in December 2019 to 0.17 (0.16, 0.18) in March 2020, and further declined to 0.07 (0.07, 0.08) in June 2020. The monthly TM rate increased from 0.7% in December 2019 to 2.7% in June 2020 (Figure). In Poisson regression of 2020 data, monthly rate for O&T visits decreased from January to March by 32% and then by another 10% from March to June 2020 (both p<0.001). The decrease was lower with increasing age by 1% (0.5%, 1.5%) per year (p<0.001), and was greater (by 16%) for patients at private clinics than public sites (p<0.05), but did not differ by insurance type, sex, race/ethnicity, or presence of VL suppression on last test (all p>0.20). The increase in TM visits (2%) did not offset the decline in office visits (26%). The HIV VL testing rate fell by 50% in the first 6 months of 2020 among patients who had VL test done in 2019 (Figure).

Conclusion: In the HOPS, the rates of office visits and HIV VL tests dropped precipitously after March 2020. The long-term implications for clinical outcomes and HIV viral suppression may not be evident at this time in the COVID-19 pandemic but HIV care sites need strategies to ensure patients maintain engagement in care and HIV laboratory monitoring.
754 WOMEN ARE LESS LIKELY TO RECEIVE DOLUTEGRAVIR-BASED FIRST-LINE ART IN SOUTH AFRICA

Jiendchi Dorward1, Yukteshwar Sookrahi2, Kelly Gate3, Thokozani Khubone1, Lara Lewis4, Christopher C. Butler1, Hope Ngobese2, Niel Garrett4

1University of Oxford, Oxford, UK; 2Theewins Municipality Health Unit, Durban, South Africa; 3Bethesda Hospital, Umhlanga, South Africa, Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

Background: South Africa has the largest ART programme globally and is currently transitioning to WHO recommended dolutegravir (DTG) first-line ART. As there were initial safety concerns for women who conceive while receiving DTG, we compared DTG rollout between sexes.

Methods: We analyzed routine data from 59 primary care clinics in KwaZulu-Natal, South Africa, between DTG introduction, in Dec 2019, to Jun 2020. Initially, DTG was prioritised for ART initiations, and women of child-bearing potential were required to sign a South African Health Products Regulatory Authority (SAHPRA) ‘acknowledgement of risk’ form. In Feb 2020, the SAHPRA form was removed, and people receiving first-line ART were also eligible for switch to DTG. We used Poisson regression models with robust standard errors, and Cox proportional hazards models, to assess the association between sex and the outcomes of 1) initiating, or 2) being switched to, first-line DTG.

Results: Of 13,395 adults newly initiated on ART, 8543 (63.8%) were women, median age was 32 years (IQR 26-38) and 6004 (44.8%) were initiated after the SAHPRA form was removed. 4460 (33.3%) initiated DTG and 8928 (66.7%) started on other regimens. DTG was prioritised for ART initiations, and women of child-bearing potential were required to sign an SAHPRA form when initiating ART. In Feb 2020, the SAHPRA form was removed and people receiving first-line ART were also eligible for switch to DTG.

Discussion: Of 13,395 adults newly initiated on ART, 8543 (63.8%) were women, median age was 32 years (IQR 26-38) and 6004 (44.8%) were initiated after the SAHPRA form was removed. Of 4460 (33.3%) who initiated DTG, 3157 (71%) were women. Median age of all adults was 32 years (IQR 26-38) and 6004 (44.8%) were initiated after the SAHPRA form was removed. 4460 (33.3%) initiated DTG and 8928 (66.7%) started on other regimens. DTG was prioritised for ART initiations, and women of child-bearing potential were required to sign a South African Health Products Regulatory Authority (SAHPRA) ‘acknowledgement of risk’ form. In Feb 2020, the SAHPRA form was removed and people receiving first-line ART were also eligible for switch to DTG.

Conclusion: Women are less likely to receive DTG-based first-line ART in South Africa, especially as recent evidence suggests lower risks from conceiving on DTG than originally feared.

755 POPULATION IMPACT OF COMMUNITY-BASED ART IN SOUTH AFRICA: A MODELING ANALYSIS

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Background: In South Africa, a lower proportion of men living with HIV are virally suppressed than women. The Delivery Optimization of Antiretroviral Therapy (DO ART) Study recently demonstrated that community-based delivery of antiretroviral therapy (ART) significantly increased HIV viral suppression compared to standard clinic delivery and eliminated the gender disparity in viral suppression. However, the impact of community-based ART delivery on population-level HIV incidence and mortality is needed.

Methods: We parameterized an HIV transmission model with data from the DO ART Study and population surveys to evaluate the impact of community-based ART delivery on HIV in KwaZulu-Natal, South Africa, a province with 27% HIV prevalence. Based on outcomes from the standard of care arm of DO ART, we estimated that 62% of women and 40% of men living with HIV are virally suppressed with clinic-based services. To represent community-based ART delivery, we modeled HIV testing campaigns that linked 90% of persons living with HIV not engaged in care to community-based ART, of whom 73% of women and 72% of men achieved viral suppression (66% suppression overall). We evaluated the 5 and 40 year impact on prevalence, incidence, and mortality of this expanded strategy compared to standard services.

Results: Under clinic-based standard of care, the projected annual HIV incidence in 2020 is 2.5% (Range using 25 best-fitting parameter sets: 1.7-3.4%) among women and 1.1% (Range: 0.7-1.7%) among men. Within 5 years, we estimate that community-based ART delivery reduces HIV incidence in women by 32.2% (Range: 29.7-34.4%). By 2060, HIV mortality in women decreases by 37.7% (Range: 34.1-41.3%). Among men, HIV mortality declines by 37.9% (Range: 32.2% (Range: 29.7-34.4%)), and HIV incidence decreases by 33.9% (Range: 28.4-42.0%) by 2060. With community-based ART, the ratio of female to male HIV prevalence narrows from 2.9 (Range: 2.3-3.3) in 2020 to 2.1 (Range: 1.7-2.4) in 2060. In total, we estimate that community-based ART delivery will avert 843,421 (Range: 667,114-1,013,236) HIV cases and 683,776 (Range: 548,375-829,802) HIV-associated deaths by 2060.

Conclusion: Community-based ART has the potential to quickly and significantly reduce mortality in men and HIV transmission to their female partners, consequently decreasing HIV incidence rates among women. Eliminating disparities in viral suppression is projected to substantially increase population health in generalized epidemic settings with high HIV prevalence.
756 COACH MPILIO: A PEER-SUPPORT INTERVENTION TO IMPROVE MEN’S ART LINKAGE & RETENTION

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Background: Men in South Africa are less likely than women to start and stay on treatment and more likely to die of AIDS-related causes. This project aimed to understand men’s barriers to linkage and retention and to co-create and evaluate solutions.

Methods: We used a combination of qualitative and quantitative research to analyze individual, social and structural barriers to linkage and retention. We first conducted in-depth interviews with purposively recruited men (n=58), analyzed thematically. We also administered a quantitative survey with randomly selected men (n=2019), analyzed using descriptive and inferential statistics. We then facilitated a series of participatory design workshops with men (n=60) and other stakeholders to identify potential solutions to identified barriers. One intervention— a peer-support model called Coach Mpilo, wherein men living well with HIV coach men still struggling with barriers— was piloted in 3 districts from March-September 2020. We evaluated the pilot using an implementation science approach, focused on assessing effectiveness in improving linkage and retention as well as implementation factors such as acceptability, feasibility, uptake, fidelity, and maintenance, using clinical data as well as interviews, focus groups and surveys.

Results: Of the 3484 men enrolled, 1387 were newly diagnosed and 2461 were previously lost-to-follow-up. 3696 men (96%) started or restarted ART during the pilot period, including 1302 (94%) newly diagnosed men and 2394 (97%) previously LTFU. Of those linked/relinked, 3511 (95%) were retained in care in 3 districts from March-September 2020. We evaluated the pilot using an implementation science approach, focused on assessing effectiveness in improving linkage and retention as well as implementation factors such as acceptability, feasibility, uptake, fidelity, and maintenance, using clinical data as well as interviews, focus groups and surveys.

Conclusions: A peer-support approach, often employed with other target populations, appears to be effective in helping men overcome barriers to HIV treatment and achieving high rates of linkage and early retention, as well as reducing HIV stigma.

757 COVERAGE OF VIRAL LOAD MONITORING DURING PREGNANCY IN SOUTH AFRICA, NATIONAL SURVEY

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Background: Maternal viral load testing for HIV positive women during pregnancy coupled with appropriate and timely interventions to achieve HIV viral suppression can improve maternal health and reduce the risk of mother-to-child transmission of HIV. Studies assessing viral load monitoring among HIV positive pregnant women in South Africa are limited. This study determined the national coverage of maternal viral load monitoring focusing on viral load testing, documentation of viral load test results, and viral suppression (viral load <50 copies/ml).

Methods: Between 1 October and 15 November 2019, a cross-sectional survey was conducted among 15-49 year old pregnant women attending antenatal care in 1589 nationally representative public health facilities. Data on antiretroviral therapy (ART) status, viral load testing, viral load result documentation, and viral suppression were extracted from medical records. Survey-based logistic regression examined factors associated with coverage of viral load testing. All analyses took into account survey design.

Results: Of 8 112 pregnant women eligible for viral load testing (905 women who initiated ART during pregnancy and received ART for at least 3 months, and 7 207 women who initiated ART before pregnancy), 81.7% received a viral load test, of which 94.1% of viral load test results were documented in the medical records (Figure 1). Among those with documented viral load test results, 74.1% were virally suppressed. A lower proportion (73.0%) of women who initiated ART before pregnancy (82.8%) were virally suppressed. A lower proportion (76.1%) among women who initiated ART during pregnancy. Viral suppression was low (56.8%) among women who initiated ART during pregnancy.

Conclusions: Most (81.7%) women received viral load testing and results documentation was high (94.1%). The low viral suppression among pregnant women initiating ART during pregnancy highlights the importance of enhanced adherence counselling and the need to fast-track the roll-out of Dolutegravir to enable achievement of more rapid viral suppression. The coverage of viral load testing could be improved further by implementing quality improvement initiatives.

Figure 1: Percent reduction in HIV incidence and HIV mortality afforded by increased viral suppression with community-based ART delivery. Shaded regions represent the range of estimates using the 25 best-fitting model parameter sets.

Figure 2: Percent reduction in HIV outcomes among Men

Figure 3: Percent reduction in HIV outcomes among Women

Figure 3: Viral load cascade among pregnant women in the national antenatal sentinel survey, 2019, South Africa
758 PREDICTORS OF VIRAL LOAD NONSUPPRESSION AT 6 MONTHS OF ART: SHARE STUDY 2015-2017
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Background: Virologic suppression is a core component of the WHO/UNAIDS 90-90-90 strategy of achieving HIV epidemic control by 2020. Uganda rolled out routine viral load (VL) testing in August 2014. This analysis aimed to determine the predictors of VL non-suppression 6 months after ART initiation in a public-sector ART programme in a resource limited setting.

Methods: Data from a 2-arm parallel, un-blinded, randomized controlled trial of nurse-initiated and monitored antiretroviral therapy (NIMART) versus clinician-initiated and monitored ART (CIMART) in HIV-infected adults in Uganda from 2015 to 2017 were analyzed. Study participants included HIV-infected, ART-naive, clinically stable adults initiated on ART at HIV treatment clinics within eight public regional referral hospitals. The primary outcome was viral load non-suppression (VL >1,000 copies/ml) at 6 months on ART. Generalized binomial regression with identity link function was used to determine statistically significant non-inferiority of viral non-suppression of NIMART from the CIMART initiated patients if the 97.5% upper CI of the difference was less than or equal to 6 % margin of error. Multivariable logistic regression was used to assess predictors of viral non-suppression. Study site was included as a fixed term in both models to control for potential confounding.

Results: Over half of the study cohort of 1,686 were female (55.3%). The overall median age was 33 years (IQR: 28-41). Almost half of the participants (49.9%) were enrolled in NIMART. Median baseline VL was 37,258 copies/ml (IQR: 7,252-118,778), with 87.8% of participants having a baseline viral load >1,000 copies/ml. The median baseline CD4 count was 343 cells/mm3 (IQR: 210-433). 23.4 % of participants had <200 cells/mm3, at baseline. Sixty percent (1,007) had a VL test done at 6 months of whom 77 (7.7%) were not virologically suppressed. NIMART was found to be non-inferior to CIMART for VL non-suppression at 6 months (Risk Difference =0.0018, 97.5% CI: -0.031-0.035). At the multivariate level, baseline CD4 cell count <200 copies/mm3, and age >24 years had a VL test at 6 months. Among those whose VL was undetectable (<1000 cp/ml), Compared to HIV-uninfected patients, those who were HIV-infected had lower BMI (mean: 26.0 vs. 28.0, p=0.05), and were less likely to be hypertensive (34.8% vs. 47.4%, p=0.01). HIV infection was not a significant predictor of severe disease (adjusted odds ratio: 1.71, 95% CI: 0.88 – 3.46), nor was it associated with 28-day mortality (adjusted hazard ratio: 0.87, 95% CI: 0.39 – 1.94).

Conclusion: In this study from a sub-Saharan African (SSA) country with a generalized HIV epidemic, HIV had no statistically significant impact on COVID-19 severity or mortality. Most of the HIV-infected population in this study were virally suppressed and this may indicate that with optimal ART and achievement of HIV viral suppression, the risk of severe disease or mortality from COVID-19 among people living with HIV (PLHIV) can be minimized. Additional studies that assess impact of COVID-19 on PLHIV not on ART are needed, as this group continues to make up a large portion of PLHIV in SSA.

759 COVID-19 SEVERITY AND MORTALITY AMONG HOSPITALIZED PATIENTS IN ZAMBIA
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1Centers for Disease Control and Prevention, Lusaka, Zambia, 2Infectious Diseases Institute, Lusaka, Zambia, 3Ministry of Health, Lusaka, Zambia

Background: Zambia-where HIV prevalence is 11.5%- is experiencing a generalized HIV epidemic, HIV had no statistically significant impact on COVID-19 severity or mortality. Most of the HIV-infected population in this study were virally suppressed and this may indicate that with optimal ART and achievement of HIV viral suppression, the risk of severe disease or mortality from COVID-19 among people living with HIV (PLHIV) can be minimized.

Methods: Data from 42 National HIV Surveillance System sites with complete laboratory reporting to CDC through December 2019 were used to determine the numbers of Asians with HIV diagnosis, the percentages linked to care within one month after diagnosis, retained in care and virally suppressed in 2018 by sex, age and transmission category.

Results: Among 786 Asians with HIV infection diagnosed in 2018, 206 (26.2%) had HIV infection classified as stage 3 (AIDS). The highest percentage of persons with HIV infection diagnosed at stage 3 (AIDS) were those 13-34 years for males and 35-54 years for females. In 2018, 662 (84.2%) were linked to care within one month after diagnosis. Males (78.4%) and females (76.5%) ≥55 years had the lowest linkage to care. Among 13,096 Asians living with diagnosed HIV infection at year-end 2017, 9,819 (75.0%) received any care, 7,681 (58.7%) were retained in care and 9,121 (69.6%) were virally suppressed in 2018. Females had lower receipt of care and retention in care than males. The lowest retention in care for males (58.8%) and females (55.2%) was among those aged 35-54 years. The lowest retention in care among those whose infection was attributed to injection drug use (IDU) was males ≤25 years (42.6%) and females 13-34 years (50.3%). The lowest retention in care among those whose infection was attributed to heterosexual contact was males ≥55 years (56.2%) and females 35-54 years (55.2%). The lowest viral suppression for males (66.8%) and females (65.2%) was among those aged ≥55 years, as well as for all transmission categories except for infection attributed to heterosexual contact among
females. The lowest viral suppression was among males (47.7%) and females (56.0%) whose infection was attributed to IDU.

**Conclusion:** HIV care outcomes among Asians were below national goals. Age and risk group appropriate strategies for Asians are needed. To address late diagnosis, HIV testing should be targeted to males 13-34 years and females 15-44 years. Increased linkage to care, receipt of care and achievement of viral suppression is needed for those ≥55 years. Improvements in retention in care for those ≥55 years are also needed.

**761 RETENTION AND VIRAL LOAD SUPPRESSION AMONG ADULTS LIVING WITH HIV ON ART IN LESOTHO**

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**Background:** Lesotho has one of the highest HIV prevalence worldwide. A national cross-sectional survey was conducted to evaluate rates of retention on antiretroviral therapy (ART) and viral load (VL) suppression among adults (≥18 years old) living with HIV (ALHIV) on ART as part of a study on HIV drug resistance (HIVDR) during 2018–2019.

**Methods:** A stratified two stage (clinics/patients) probability proportional to size (PPS) sampling design was used to achieve representative samples from three targeted ALHIV population. Sample 1 includes ALHIV who initiated ART 15–27 months prior to the survey. Sample 2 and 3 includes current ALHIV who have been on ART for 9–15 months and for ≥48 months respectively. Sample 1 was used to evaluate retention rates (proportion of patients who had a clinic visit within 12±3 months after ART initiation excluding documented out-transfers or known dead) by reviewing patient charts. Laboratory tests for HIV VL were conducted for ALHIV in sample 2 and 3 to evaluate VL suppression rate (proportion of patients with VL<1000 copies/ml). Proportions and 95% confidence intervals are used to estimate prevalence. Chi-square test and logistic regression models were used to investigate correlates of retention and/or VL suppression. Sampling weights were applied to and PPS design was accounted for all analysis.

**Results:** Among 501 ALHIV in sample 1, 60% were female, median age was 37 years (IQR: 29–46 years). The overall retention rate was 75.3% (95% CI: 67.5–83.1%). While district differences in retention rates were observed (p<0.001), retention rates did not differ by sex or age groups. VL suppression rates among ALHIV on ART for 12 months (n=385) and ≥48-mo (n=490) cohorts were high at 93.4% (95% CI: 90.2–95.6%) and 92.1% (95% CI: 88.5–94.6%), respectively. While VL suppression did not differ by sex, younger age groups had lower VL suppression at 12 months (p<0.001) and 48 months (p<0.001). In the 12-month cohort, older age groups (adjusted odds ratios [AOR]; 95% CI): 25–44 years: 1.4 (1.1, 1.6), ≥45yrs: 2.5 (1.6, 4.0, 5.1), 18–24 years as reference) and ALHIV with no prior ART exposure (AOR(95%CI): 18.2 [11.3–28.1]) had higher VL suppression rates in logistic regression model. Few ALHIV (2 in sample 2 and 3 in sample 3) were on 2nd line ART regimen and all had VLS.

**Conclusion:** While VL 5 rates among ALHIV retained in care are high, retention rate was sub-optimal. Younger age groups and ALHIV with prior ART exposure require tailored intervention to improve VLS.

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**Table: Rates of retention and viral load suppression among adults living with HIV on antiretroviral therapy in Lesotho, 2018–2019 survey**

<table>
<thead>
<tr>
<th>Retention at month 12, n=490</th>
<th>Viral load suppression rate among 12-month cohort, n=385</th>
<th>Viral load suppression rate among ≥48 months cohort, n=490</th>
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</thead>
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<tr>
<td>Sample, %</td>
<td>Retention rate, %</td>
<td>Sample, %</td>
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<td>Total</td>
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</tbody>
</table>

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**IMPACT OF IMMEDIATE ART FOR PATIENTS WITH KNOWN HIV EXPERIENCING A GAP IN HIV CARE**

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**Background:** Immediate antiretroviral therapy (ART) at HIV diagnosis is endorsed by DHHS guidelines. Less is known about the impact of immediate ART for individuals with known HIV who are either not in care or facing a lapse in care.

**Methods:** Since 2016, San Francisco AIDS Foundation (SFAF)/Magnet, a nurse-led, community-based sexual health and wellness clinic without on-site primary care, has provided immediate interim ART, support services, and care linkage to individuals with known HIV who are not in care or facing a lapse in care. Data on services needed/rendered, ART, and viral load (VL) were abstracted from the medical record and supplemented by pre and post-visit VL data from the San Francisco Department of Public Health. We calculated the proportion of individuals who achieved viral suppression (VS) as well as those found to maintain VS on first VL obtained after their SFAF encounter; we considered those with missing follow up VL as all suppressed or all unpressed in a sensitivity analysis (SA).

**Results:** Between October 2016 and March 2020, 260 individuals with known HIV presented to SFAF needing services to support HIV management. Individuals had median age of 34 years and were 97% cis-men, 65% MSM, 33% Hispanic, 8% Black. At presentation, 189 (73%) were on ART, 61 (23%) were ART experienced but not taking ART, 10 (4%) were ART naive. The most common reasons for presenting were loss of insurance 58%, relocation 37%, STD services 17%, or a problem with long-term HIV provider 11%. The most common services provided were ART prescription 92%, lab services to maintain benefits 66%, linkage support 63%, and medical or pharmacy benefits support 60%. Of 239 individuals requesting ART prescription, 99.6% received one: 93% same-day and 97% within 7 days. Of 143 individuals without evidence of VS at baseline, 75% (SA 60%-80%) were suppressed on first follow up VL. Of 117 individuals with VS at baseline, 94% (SA 80%-95%) sustained VS. Of ART naïve individuals, 90% accepted ART initiation, 89% of whom subsequently demonstrated VS.

**Conclusion:** A nurse-led sexual health center without on-site primary care can successfully prevent viral load rebound in individuals facing care lapses and achieve suppression in those never or no longer in care. Given the majority of forward HIV transmission is from those with known HIV who are not virologically suppressed, expanding the immediate ART paradigm to include those previously diagnosed is an important tool for improving both individual and population health.

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**DIFFERENCES IN SEXUAL BEHAVIORS BEFORE AND AFTER UNIVERSAL TEST AND TREAT IN UGANDA**

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**Background:** Universal Test and Treat (UTT) with antiretroviral therapy (ART) reduces HIV morbidity and mortality rates and the risk of transmission. The 2015 World Health Organization ART initiation guidelines called for implementation of universal ART as part of Universal Test and Treat programs (UTT). However, there has been concern that increased use of ART could alter HIV risk perception, and lead to risk compensation, which could potentiate the continued spread of the epidemic especially in HIV hotspot areas like the fishing communities. We assessed the impact of universal test and treat on sexual behaviors in the Rakai Community Cohort Study (RCCS).

**Methods:** We used RCCS data from 2011-2018 for consenting participants aged 15–49 years. The main exposure period was prior to UTT initiation 2011/2013 (R015) versus after UTT from 2014-2018. Risky sexual behaviors were inconsistent condom use with any sexual partner in the past 12 months, multiple sexual partners, alcohol use before sexual intercourse and unknown HIV status of sexual partner. We stratified analysis by fishing communities and non-fishing communities. Frequency distributions were used to estimate
prevalence of risk behaviors and chi square statistic to assess significant differences before and after UTT initiation periods. A mixed-effects logistic model was fitted to estimate the association of UTT on selected risky sexual behaviors. We adjusted for age in years, gender, occupation, highest education level, marital status, and community type.

Results: A total of 14,193 participants were enrolled in this study. Of these, 25% (3556) were from fishing communities. Inconsistent condom use was the most of selected sexual risk behaviors, both before and after initiation of UTT (90.0% and 91.7% respectively) and was not significantly different post-UTT (P=0.327). After UTT, there was 21.0% decrease in the proportion of participants who lacked knowledge about their partners’ HIV status (from 54.9% before UTT to 33.9% after UTT; P<0.001). While prevalence of inconsistent condom use was lower in the fishing communities compared to the non-fishing communities before and after UTT.

Conclusion: We found no evidence of risk compensation among sexual behaviors in the era of UTT implementation, though prevalence of the selected sexual behaviors remains consistently high in fishing compared to non-fishing communities. UTT has resulted in increasing proportion of persons knowing the HIV status of their sexual partners.

764 APPLYING MACHINE LEARNING TO ROUTINE HIV DATA: PREDICTING MISSED CLINIC VISITS

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Background: To optimize South Africa’s HIV response and reach targets of 95% tested, 95% treated, and 95% virally suppressed, numbers initiating and successfully remaining on antiretroviral therapy (ART) must increase. While much effort and resources have been focused on tracing those lost to follow-up and returning them to care, little prior work has successfully addressed identifying those most at risk of poor treatment outcomes while they are still engaged in care.

Methods: We applied machine learning and modelling algorithms to routinely-collected ART patient data from the SLATE I and SLATE II trials which evaluated same-day ART initiation in 2017-18. Our primary outcome was the probability of a patient’s attending the next scheduled clinic visit. Three classification algorithms were tested: logistical regression, random forest and Adaboost classifier. Demographic, clinical, socioeconomic, care-seeking behavioural (visit patterns), and laboratory data points were investigated as potential predictor variables. Predictions were scored for accuracy against known outcomes in the source dataset.

Results: Data from 916 patients who initiated ART were analyzed in 7 models using multiple combinations of input features and classification algorithms. The best model achieved 90% specificity and 69% accuracy and an area under the curve of 0.68 for attendance at next scheduled visit, suggesting that the model correctly anticipated whether a scheduled visit would be attended for 2 out of 3 patients. Prior patient behavior and treatment history were important in predicting visit attendance, while demographic and socioeconomic characteristics were useful in creating patient profiles to stratify groups (Table 1) into risk profiles. Compared to puntual attenders with social support, a patient who is late to previous appointments was 1.69 times more likely to miss a scheduled visit, while an employed patient aged 18-29 with a scheduled visit near payday was 1.21 times more likely to miss the next visit.

Conclusion: If facilities could identify patients at higher risk of loss to care while the patient is still active, micro-targeted interventions could be implemented more efficiently and pro-actively, rather than only after a patient is lost. Predictive models may allow providers to combine existing medical record data on prior behavior and treatment history with a simple demographic and socioeconomic questionnaire to assess individual risk and offer personalized, differentiated care.
ECONOMIC EVALUATION OF DIFFERENTIATED SERVICE DELIVERY OF HIV TREATMENT IN ZIMBABWE

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Background: Zimbabwe has >1.4 million people living with HIV, of whom 85% are on antiretroviral treatment (ART), but the country has not yet achieved the UNAIDS 95-95-95 targets, and further expansion of its treatment program will require more efficient use of existing resources. One promising strategy for reducing resource utilization, and thus cost, per patient treated is multi-month dispensing of medications. We evaluated the costs to providers of community-based, multi-month ART delivery models in Zimbabwe.

Methods: We used resource and outcome data from a cluster-randomised non-inferiority trial of different models of differentiated service delivery models, targeted to patients stable on ART. The trial compared retention in care at 12 months in care for 3-month facility-based care (standard of care) with that for 3- and 6-month community ART refill groups (CARGs), which dispensed medications for 3- and 6-month intervals at a time, respectively. Using local unit costs, we estimated the annual cost of providing HIV care and treatment per patient from the provider (health system) perspective across 12 months after entry into each study arm. Costs are reported in 2018 USD.

Results: In the trial, retention at 12 months was 91.0% in the 3-month facility visit arm (SOC), 93.3% in the 3-month CARG arm, and 93.6% in the 6-month CARG arm. The total average annual costs of HIV treatment per patient were $183 (standard deviation $32), $190 ($32), and $179 ($35) in each of the three arms, respectively. The annual cost per patient was dominated by medications (87% in 3-month facility-based care, 83% in 3-month CARG, 92% in 6-month CARG), followed by facility visits (8%, 7%, 5%, respectively) and viral load (5%, 2%, 2%, respectively). If those in the 6-month CARG arm received a similar number of viral loads on average as in the other two arms, the cost-savings of the 6-month CARG arm disappear.

Conclusion: While the 3- and 6-month CARG arms were more effective at retaining patients in care, they had similar costs from the provider perspective as 3-month facility-based care. Costs to the patients should be evaluated in future work, given that lower patient costs can support long-term adherence. Given the cost-neutrality of the CARG models and improved patient outcomes, these models should be prioritized for further scale-up.

<table>
<thead>
<tr>
<th>Resource utilization</th>
<th>Conventional care (3-monthly visits)</th>
<th>3-month CARG</th>
<th>6-month community distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility visits (mean [SD])</td>
<td>3.29 (0.89)</td>
<td>2.68 (0.61)</td>
<td>2.65 (0.57)</td>
</tr>
<tr>
<td>DOS interactions (mean [SD])</td>
<td>4.49 (1.41)</td>
<td>1.71 (1.50)</td>
<td></td>
</tr>
<tr>
<td>Viral load (mean [SD])</td>
<td>0.675 (0.565)</td>
<td>0.732 (0.634)</td>
<td>0.382 (0.452)</td>
</tr>
<tr>
<td>ART months dispensed (mean [SD])</td>
<td>13.4 (1.96)</td>
<td>13.4 (1.81)</td>
<td>13.4 (1.32)</td>
</tr>
</tbody>
</table>

Methods: Cross-sectional survey among stable ART patients (n=390, clinic-based; n=251, club-based care). For each group, we collected socio-demographic, income, and expenditure data. We estimated patient-incurred costs - direct and indirect costs. Direct costs included out-of-pocket expenditures. Indirect costs included income loss due to time spent during transport, accessing services, and off work during illness. Cost drivers were assessed in multivariate regression models.

Results: Overall, costs were significantly higher among clinic participants. Costs (USD) per year for clinic vs club were: 117 vs 41.7 (p<0.001) for direct costs, 20.2 vs 7.71 (p<0.001) for indirect costs and 31.8 vs 11.9 (p<0.001) for total costs. Time spent accessing care and time spent in illness (hours/year) were 38.3 vs 13.8 (p<0.001) and 16.0 vs 6.69 (p<0.001), respectively. The main cost drivers included transportation (clinic vs club: 67.7% vs 44.1%) for direct costs, and income loss due to time spent accessing care (clinic vs club: 60.4% vs 56.7%) for indirect costs. Factors associated with higher total costs among patients attending clinic services were higher education level (coefficient [95% confidence interval]) 13.0 (0.8, 25.3)), formal employment (30.9 (11.4, 50.5)) and higher income (43.8 (35.4, 52.2)); among those attending the clubs only higher income (9.02 (4.12, 13.9)) was significant. The percentage of households classified as having had catastrophic expenditures in the last year was low but significantly higher among clinic participants (10.8% vs 5.18%, p=0.014).

Conclusion: Costs incurred by patients accessing DSD in the community are significantly lower compared to those accessing standard clinic-based care. DSD models provide promising benefits which could improve access and could potentially catalyze the attainment of global targets especially in resource limited settings.

CHANGES IN HIV DIFFERENTIATED CARE UTILIZATION DURING THE COVID-19 PANDEMIC IN ZAMBIA

Youngji Jo1, Sydney Rosen3, Amy Huber4, Muya Mwansa5, Mpande Mwenechanya1, Priscilla Lumano-Mulenga1, Bevis Phiri6, Hilda Shakkwele3, Prudence Haimbe3, Brooke Nichols2

1Boston University, Boston, MA, USA, 2Health Economics and Epidemiology Research Office, Johannesburg, South Africa, 3Ministry of Health, Lusaka, Zambia, 4Clinton Health Access Initiative, Lusaka, Zambia

Background: Differentiated service delivery (DSD) models aim to lessen the burden of HIV treatment on patients and providers in part by reducing requirements for facility visits and extending dispensing intervals. With the advent of the COVID-19 pandemic, minimizing patient contact with healthcare facilities and other patients, while maintaining treatment continuity and avoiding loss to care, has become more urgent, resulting in efforts to increase DSD. In March, the Zambian Ministry of Health urgently promoted the 3- and 6-monthly intervals for patients on antiretroviral treatment (ART). We assessed the extent to which DSD coverage and ART dispensing intervals have changed during the COVID-19 pandemic in Zambia.

Methods: We used patient data from SmartCare, Zambia’s electronic medical record system, for 737 health facilities, representing about 3/4 of all ART patients nationally, to compare the numbers and proportional distributions of patients enrolled in DSD models and the average duration of drug dispensing
between February 15, 2020, and October 30, 2020, 8 months after the first recorded COVID-19 case in Zambia on March 18, 2020.

**Results:** As expected, participation increased for all DSD models. The number of patients presently enrolled in a DSD model increased by 60% between February and October, from 134,652 (18% coverage) to 215,947 (29% coverage), though remaining below one third of all patients. Home ART delivery saw the greatest percent increase in utilization (240%), while community adherence groups experienced the smallest change (18%), potentially a reflection of efforts to discourage group models due to COVID-19 transmission risk. Although 6-month dispensing is Zambia’s national policy for stable patients, the proportion of patients receiving 6-month supplies fell from 57% to 49%, while the proportion of patients receiving a 1, 2, or 3-month supplies rose. The shortening of dispensing intervals is primarily due to patients switching temporarily back from Tenofovir Lamivudine Dolutegravir (TLD) to Tenofovir lamivudine Efavirenz (TLE) to mitigate threats of TLD global supply chain.

**Conclusion:** The months of the COVID-19 pandemic showed increased participation in DSD models for stable ART patients in Zambia but shorter dispensing intervals. Efforts to eliminate obstacles to longer dispensing intervals should be prioritized to achieve the expected benefits of DSD models and minimize COVID-19 risk.

### Table. Numbers and proportional distributions of patients enrolled in differentiated service delivery models in Zambia

<table>
<thead>
<tr>
<th>Indicator</th>
<th>As of February 15 2020</th>
<th>As of October 30 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% of total patients on ART (coverage)</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>of all patients in DSD models</td>
</tr>
</tbody>
</table>
| proportion of patients in coverage | 771 TRANSMISSION IMPACT OF PrEP UPTAKE IN URBAN CENTERS IN BRAZIL: A MODELING STUDY

**Objectives:** To evaluate the transmission impact of pre-exposure prophylaxis (PrEP) uptake in 13 urban centers in Brazil using a modeling approach.

**Methods:** A mathematical model was developed to analyze the impact of PrEP uptake on the HIV epidemic in Brazil. The model incorporates epidemiological, behavioral, and population-specific parameters. The transmission impact was assessed using different scenarios of PrEP uptake.

**Results:** The model projections indicate that a substantial increase in PrEP uptake could significantly reduce the number of HIV infections in Brazil. The impact is more pronounced in urban centers with higher prevalence of HIV.

**Conclusion:** The implementation of PrEP programs in urban centers in Brazil has the potential to significantly curb the transmission of HIV, thereby slowing the epidemic. Continued efforts to increase PrEP uptake are crucial for achieving this goal.

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**770 C2C: DIFFERENTIATED CARE INCREASES HIV CARE AFTER PRISON RELEASE IN SOUTH AFRICA**

**Christopher Hoffmann, Jill Owczarzak, Stefan Baral, Colleen Hanrahan, Tonderai Mabuto**

1. Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2. Johns Hopkins University School of Public Health, Baltimore, MD, USA, 3. Aureum Institute, Johannesburg, South Africa

**Background:** Globally a large proportion of people with HIV (PWHR) pass through correctional facilities. During incarceration, PWHR often achieve an undetectable viral load. Post release, retention in care and ART is suboptimal, with estimated 34% retention in South Africa. We built upon formative work among reentrants in South Africa and the experience from the United States to develop and test in a randomized trial a multi-level intervention to increase linkage to and retention in HIV care during community reentry in South Africa.

**Methods:** Our intervention was built on the differentiated care concept of community adherence clubs with group meetings. Groups met every two weeks for 6 months to two hours with facilitated interaction by a peer (reentrant living with HIV) and a social worker and included peer bonding, an interactive curriculum, ART provision every two months, and referrals to services not able to be provided through this group. We refer to this intervention as a transitional community adherence club (TAC). We enrolled and randomized participants 2:1 in the TAC or care as usual (CAU) (a referral letter from the correctional facility at release). We had follow-up visits with all participants post-release at 1 week and 1, 3, and 6 months. We compared linkage within 90 days of release by arm.

**Results:** We enrolled and randomized 175 incarcerated individuals (116 in TAC and 59 in CAU). 95% were men, the median age was 33 years, the median duration of incarceration was 10 months, and 32% reported opioid use. Among those with either participant or family contact 41 (23%) were reported to be living on the streets, 19 (11%) were re-incarcerated, 3 (2%) had died, and 86 (49%) reported that they were in care within 90 days. Among TAC participants, 67 (58%) were in care compared to 39 (32%) of CAU (chi square p=0.001).

**Conclusion:** We adapted an existing differentiated care model to successfully increase retention in care during community reentry in South Africa. This is the only intervention reported on care retention during reentry in Africa and one of the few globally to demonstrate success. We hypothesize that the use of peer support and a multilevel intervention contributed to the success. Differentiated care models may have an important role for a variety of populations who are underserved by traditional HIV care models, including community reentrants.
increasing the uptake of publicly funded, daily, oral tenofovir/emtricitabine (TDF/FTC) for pre-exposure prophylaxis (PrEP).

**Methods:** We used the Cost-Effectiveness of Preventing AIDS Complications (CEPC) model to assess the impact of increasing PrEP uptake to different levels (range 10–60%) across various timelines (within 1–4 years) in a cohort of adult (≥18 years) GBM without HIV. We used local studies, national data, and the international literature to represent the HIV epidemic in three Brazilian cities: Rio de Janeiro (Southeast), Salvador (Northeast), and Manaus (North). Age-stratified HIV incidence rates were highest in Rio de Janeiro (4.3/100 person-year (PY), 2.5/100PY in Salvador and 1.4/100PY in Manaus); PrEP efficacy was 96%, and adherence was 74%. Outcomes included HIV infections, with and without PrEP, over 5 and 10 years. In sensitivity analyses, we examined how results varied with changes in adherence to PrEP (range 50–85%), drop-out rates (range 0–25%/year), and age at initiation of PrEP (21–33 years). We also estimated the PrEP uptake level needed to reach a 75% incidence reduction in 5 years.

**Results:** We found that a PrEP intervention achieving 10% uptake among GBM within 60 months could avert 501 infections in Rio de Janeiro (Salvador: 161, Manaus: 119) by 5 years. An intervention achieving 60% uptake among GBM within 24 months would avert ~10-times as many infections (35.9% of new HIV infections). GBM within 60 months could avert 501 infections in Rio de Janeiro (Southeast), Salvador (Northeast), and Manaus (North). Age-stratified HIV incidence rates were highest in Rio de Janeiro (4.3/100 person-year (PY), 2.5/100PY in Salvador and 1.4/100PY in Manaus); PrEP efficacy was 96%, and adherence was 74%. Outcomes included HIV infections, with and without PrEP, over 5 and 10 years. In sensitivity analyses, we examined how results varied with changes in adherence to PrEP (range 50–85%), drop-out rates (range 0–25%/year), and age at initiation of PrEP (21–33 years). We also estimated the PrEP uptake level needed to reach a 75% incidence reduction in 5 years.

**Conclusion:** Increased oral PrEP uptake in Brazil would substantially decrease HIV transmission over the next 5 to 10 years. PrEP uptake would need to be extremely high to achieve a proposed target of 75% incidence reduction within 5 years.

**Results:**

- **5 years.**
  - Extremely high to achieve a proposed target of 75% incidence reduction within 5 to 10 years. PrEP uptake would need to be 80%.
  - A 50% drop-out rate decreased the transmission impact of PrEP.
  - To reach 75% incidence reduction in 5 years, a PrEP intervention would need to achieve 80% adherence to PrEP over 5 and 10 years.
  - In sensitivity analyses, we examined how results varied with changes in adherence to PrEP (range 50–85%), drop-out rates (range 0–25%/year), and age at initiation of PrEP (21–33 years).
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- Increased oral PrEP uptake in Brazil would substantially decrease HIV transmission over the next 5 to 10 years. PrEP uptake would need to be extremely high to achieve a proposed target of 75% incidence reduction within 5 years.

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**Conclusion:**

- Increased oral PrEP uptake in Brazil would substantially decrease HIV transmission over the next 5 to 10 years. PrEP uptake would need to be extremely high to achieve a proposed target of 75% incidence reduction within 5 years.
774 INVESTMENT IN A KEY POPULATION HIV PROGRAM IN NIGERIA: A COST-EFFECTIVENESS ANALYSIS
Moses Katbi, Adeseyiwa Adegboyi, Helina Meri, Kent Klintdor, Adeoye Adegboyi, Abdulmalik Abubakari, Amal Akhunukwu Ukareche, Abdulmasad Salihu, Wole Fajemisin, Segun K. Fatoyinbo, Rachel Goldstein

**Background:** Key populations (KPs) - including men who have sex with men (MSM), female sex workers (FSW), people who inject drugs (PWID), and transgender people (TG) account for 32% of new HIV infections in Nigeria. Investing in KP programs is critical to achieving HIV epidemic control. We increased the HIV case-finding targets for KPs in two geographic areas while increasing investment in human and financial resources. This study looked at the cost effectiveness of increasing investment for the KP program.

**Methods:** A multi-centric retrospective study covering a six-month period divided into pre-surge (October 2019-February 2020) and surge (March-April 2020). We implemented routine HIV services during the pre-surge, while in the surge period, human and financial resources was increased in line with an increased program target. Number of peer navigators was increased from 66 (pre-surge) to 226 (surge) and budget from $47,614 (pre-surge) to $112,504 (surge). Target for HIV positive clients was also increased from 187 (pre-surge) to 1,914 (surge). We conducted a Cost effectiveness analysis (CEA) based on clients reached, tested, positives identified and linked to treatment. Chi square and Spearman’s rank order correlation was used to determine the relationship between every $1,000 spent per period and the outcome variables.

**Results:** Pre-surge, $47,614 was spent with 66 peer navigators engaged, reaching 3,380 KPs at a cost per target (CPT) of $14, testing 1,200 KPs (CPT=$40), and identified 138 new positive clients (CPT=$345) with 126 linked to treatment (CPT=$375) giving a linkage rate of 91%. For surge, $112,504 was spent with 226 peer navigators reached 23,060 KPs (CPT=$5), tested 15,554 (CPT=$7), and identified 1,811 new positive KPs (CPT=$63), with 1,757 linked to treatment (CPT=$64) giving a linkage rate of 97%. CEA shows that for every $1000 spent at pre-surge, 71 KPs were reached, 25 tested with 3 positive cases identified. With surge, for every $1000 spent, 200 clients were reached, 140 tested with 16 new positive identified. Chi square test of independence showed the relationship between project period and achievement/$1000 spent was statistically significant ($2 = 7.768, df = 2, p = 0.021). Spearman rank order correlation shows a positive correlation between project period and achievement/$1000 spent (rs = 0.129, p = 0.006).

**Conclusion:** Increasing investment for KPs is a valuable approach towards achieving HIV epidemic control among a group disproportionately infected with HIV.

For the total patient cohort, our model had an AUC of 0.96 with an APS of 0.45. For the female-only cohort, the AUC was 0.95 with an APS of 0.27. We determined the most predictive variables of HIV risk based on their individual SHAPE values (Fig. 1). Globally, the most predictive variables for both cohorts were race, age, and zip code. The most impactful predictors for the total cohort were hepatitis A (reactive IgM), receipt of IM ceftriaxone, and receipt of IM penicillin, and for the female-only cohort were hepatitis A, buprenorphine or methadone usage, and history of domestic or sexual abuse.

**Conclusion:** Our models had high AUCs and acceptable precision scores in general and for women, reflecting their ability to identify individuals at increased risk for incident HIV diagnosis in a Southern health system. This is the first EHR-prediction model with acceptable performance for women and holds promise to optimize PrEP implementation in the South.

**Figure 1.**

Shapley Additive Explanation (SHAPE) Values of Individual Electronic Health Record Variables

<table>
<thead>
<tr>
<th>Feature Importance: Total Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
</tr>
<tr>
<td><strong>Low</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature Importance: Female Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
</tr>
<tr>
<td><strong>Low</strong></td>
</tr>
</tbody>
</table>

775 PROJECTED IMPACT OF TARGETED PrEP AND VIRAL SUPPRESSION AMONG MSM IN 4 US CITIES
Melissa Schnure, Parastu Kasaie, David Dowdy, Maunank Shah, Anthony T. Fojo
1 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** The announcement of the United States’ Ending the HIV Epidemic (EHE) initiative in 2019 articulated a comprehensive strategy to end HIV in high-burden jurisdictions across the country. However, the localized nature of
the HIV epidemic in the US suggests that decisions regarding specific priority interventions and key populations must be made at the local level.

Methods: We applied Johns Hopkins Epidemiological and Economic Model (JHEEM), a dynamic, compartmental model of HIV epidemic in US, to evaluate the relative benefits of increased pre-exposure prophylaxis (PrEP) coverage versus improved viral suppression in young (age <35) men who have sex with men (MSM) versus all MSM. We selected four metropolitan statistical areas (MSAs) for comparison: Los Angeles, New York City, Atlanta, and Baltimore. In each MSA, we compared scenarios to improved PrEP coverage (proportion of individuals at risk for HIV infection who are prescribed and adherent to PrEP and tested for HIV every three months) and improved viral suppression at three levels, assuming scale-up from 2021-2022, with continuation from 2022-2030. Our primary outcome was the MSA-wide reduction in HIV incidence by 2030, evaluated as the mean across 200 independent model simulations.

Results: Across the four MSAs, scaling up PrEP among young MSM yielded large reductions in incidence, while the marginal impact of expanding PrEP interventions to all MSM was modest. The possible benefit of improved suppression, on the other hand, was maximized by scale-up among all MSM more broadly. For example, in Los Angeles, 25% PrEP coverage among young MSM (versus all MSM) reduced ten-year HIV incidence by a projected 38% (versus 49%), whereas increasing suppression to 85% provided a 26% (versus 51%) reduction. Overall, an optimistic strategy of focused PrEP among young MSM (25% coverage) plus broad increases in suppression among all MSM (85% suppression) could lead to a 58-70% reduction in HIV incidence by 2030, while an aspirational strategy of 50% PrEP coverage among young MSM combined with 90% suppression among all MSM was nearly sufficient to meet EHT targets (74-83% reduction in HIV incidence by 2030).

Conclusion: The impact of improved PrEP coverage and viral suppression varies substantially at the local level, but model projections suggest that a strategy targeting PrEP to young MSM while improving suppression levels among MSM in care more broadly could be effective.

Results: We recruited 1179 participants in Montreal, 517 in Toronto and 753 in Vancouver. The RDS-adjusted HIV prevalence was 14.2% (95% CI:11.1-17.2%) in Montreal; 22.1% (95% CI:12.4-31.8%) in Toronto, and 20.4% (95% CI:14.5-26.3%) in Vancouver (p<0.001). Of participants with confirmed HIV infection, 3.3% were previously undiagnosed in Montreal, 3.2% undiagnosed in Toronto and 0.2% in Vancouver (p=0.154). In Montreal, 87.6% of GBM living with HIV were receiving antiretroviral therapy (ART) and 10.6% had an unsuppressed VL, in Toronto, 82.6% were receiving ART and 4.0% were unsuppressed; in Vancouver, 88.5% were receiving ART and 2.6% were unsuppressed (p<0.001 for receiving ART and 0.009, for unsuppressed VL). Multivariable modelling demonstrated that participants in Vancouver (adjusted odds ratio [AOR]=0.23; 95% CI 0.06-0.82), but not Toronto (AOR=0.27; 95% CI 0.10-0.73), had lower odds of unsuppressed VL, compared to Montreal, as did older participants (AOR 0.93 per year; 95% CI 0.89-0.97), those at high-risk for hazardous drinking (AOR=0.19; 95% CI 0.05-0.70), those with a primary care provider (AOR=0.11; 95% CI 0.02-0.57), and those ever diagnosed with other STIs (AOR=0.12; 95% CI 0.04-0.32).

Conclusion: GBM living in Montreal, Toronto and Vancouver are highly engaged in HIV testing and treatment and all three cities have largely achieved the 90-90-90 targets for GBM. Nevertheless, we identified disparities which can be used to identify GBM who may require additional interventions to maximize HIV treatment benefits, in particular younger men and those who are without a regular primary care provider.

Table: Logistic regression analysis of factors associated with having a VL <200 copies/mL among 421 participants living with HIV in the Engage Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>City</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montreal</td>
<td>0.94 (0.81-1.10)</td>
<td>0.90 (0.74-1.09)</td>
</tr>
<tr>
<td>Toronto</td>
<td>0.69 (0.55-0.88)</td>
<td>0.71 (0.57-0.89)</td>
</tr>
<tr>
<td>Vancouver</td>
<td>0.66 (0.53-0.84)</td>
<td>0.70 (0.56-0.87)</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>0.43 (0.29-0.64)</td>
<td>0.45 (0.30-0.67)</td>
</tr>
<tr>
<td>Confirmed</td>
<td>0.71 (0.55-0.91)</td>
<td>0.74 (0.57-0.96)</td>
</tr>
<tr>
<td>Never tested</td>
<td>0.82 (0.63-1.06)</td>
<td>0.84 (0.65-1.08)</td>
</tr>
<tr>
<td>High-risk behavior</td>
<td>0.67 (0.49-0.92)</td>
<td>0.70 (0.51-0.96)</td>
</tr>
<tr>
<td>AUDIT C</td>
<td>0.69 (0.54-0.89)</td>
<td>0.73 (0.57-0.93)</td>
</tr>
</tbody>
</table>

776 HIV CASCADE OF CARE AMONG MEN WHO HAVE SEX WITH MEN IN CANADA’S 3 LARGEST CITIES

David Moore1, Zishan Cu1, Shyan Skakoon-Sparling2, Jordan Sang3, Justin Barath4, Lu Wang5, Nathanch Lachowsky6, Joseph Cox7, Gilles Lambert8, Syed W. Noor9, Daniel Grace10, Jody Jollimore11, Lu Wang10, Zishan Cui1, Shayna Skakoon-Sparling2, Jordan Sang3, Justin Barath4, Lu Wang5, Nathanch Lachowsky6, Joseph Cox7, Gilles Lambert8, Syed W. Noor9, Daniel Grace10, Jody Jollimore11, Lu Wang10, Zishan Cui1

1British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada, 2McGill University Health Centre, Montreal, Canada, 3University of Toronto, Toronto, Canada, 4University of Victoria, Victoria, Canada, 5McGill University, Montreal, Canada, 6McGill University Health Centre, Montreal, Canada, 7University of Toronto, Toronto, Canada, 8Community-Based Research Centre, Vancouver, Canada

Background: Treatment as prevention strategies have been variously applied across provinces in Canada. We estimated HIV care cascade indicators and correlates of unexpressed viral load (ULV) among gay, bisexual and other men who have sex with men (GBM) recruited in Montreal, Toronto and Vancouver.

Methods: Sexually active GBM, aged ≥16 years, were recruited through respondent-driven sampling (RDS) from February 2017 to August 2019. Participants completed a Computer-Assisted Self-Interview and tests for HIV and other sexually transmitted infections (STIs). We conducted bivariate analyses comparing RDS-adjusted proportions across cities. The p values generated refer any significant difference across the three cities. We used multivariable logistic regression to examine factors associated with having a VL <200 copies/mL with data pooled from all three cities.

777 REDUCTION IN INFECTIOUS SARS-CoV-2 IN TREATMENT STUDY OF COVID-19 WITH MOLNUPRIVAR

Wendy P. Painter1, Timothy Sheahan2, Ralph Baric3, Wayne Holman1, John Donovan4, Lei Fang1, Paul Alabanza5, Joseph J. Eron6, Erin Goeker7, Robert Coombs8, William Fischer9

1Ridgeback Biotherapeutics, LA Miami, FL, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3Pharstat Inc, Raleigh, NC, USA, 4University of Washington, Seattle, WA, USA

Background: The emergence of SARS-CoV-2 viral variants threatens current anti-viral and preventative strategies, including monoclonal antibodies and vaccines. Critically, the limited supply of vaccines and the complex logistics of the delivery of infusion-based therapies herald the need for an easily produced, distributed, and specific direct-acting antiviral for COVID-19 that limits progression of illness and ideally prevents transmission.

Methods: The efficacy of molnupiravar was evaluated in a double-blind, randomized, placebo-controlled, Phase 2 dose-range finding study using realtime polymerase chain reaction (RT-PCR) and virus isolation was conducted at 11 study sites in the U.S.

Participants were randomized if they had signs or symptoms of COVID-19 within 7 days, and a positive SARS-CoV-2 RT-PCR within 4 days of enrollment. Initially, participants were randomized in a 1:1 ratio to receive 200 mg molnupiravar or placebo twice daily for 5 days. Subsequently, in the dose-range finding portion of the study, participants were randomized in a 3:1 ratio to receive 200, 400, or 800 mg molnupiravar or placebo twice daily for 5 days. Nasopharyngeal swabs were analyzed from 175 subjects at enrollment, Day 3, and Day 5 for SARS-CoV-2 infectivity. Samples were stored at 4°C for up to 72 hours, shipped refrigerated, aliquoted, and stored at -80°C until testing. Vero E6 cell monolayers were infected with the sample for 1 hour. Culture media was analyzed for viral load at 2 and 5 days post-infection by RT-PCR.

Results: Seventy-eight (65%) participants, median 4.62 days (min. 1.40, max. 7.54) from symptom onset, had a positive SARS-CoV-2 culture at enrollment (52 on active and 26 on placebo). The percentage of participants with a positive viral culture at enrollment who were positive on Day 3 was 20.4% on active and 28% on placebo (p = 0.56). At day 5, 24% of placebo participants were culture-
positive compared to none treated with molnupiravir ($p = 0.001$). Between treatment, comparisons were performed using Fisher's exact test.

**Conclusion:** This is the first demonstration of reduced infectiousness by antiviral therapy in people with SARS-2 infection. This simple, short-course oral therapy may benefit individuals and public health and is unlikely to be impacted by spike-protein variants.
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