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Selected Highlights of the 2023 Conference on Retroviruses and Opportunistic Infections (Part 2)

30th CROI 2023
Conference on Retroviruses and Opportunistic Infections

Topics in Antiviral Medicine™

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- Recognize and diagnose the metabolic and neuropsychiatric complications in people with HIV, including cardiovascular disease, cancer, obesity, and frailty
- Analyze the important new data presented at the 2023 Conference on Retroviruses and Opportunistic Infections on basic virology science in HIV, including HIV-1 persistence and reservoir studies
- Describe the clinical presentation, diagnosis, and treatment options for tuberculosis in adults and children with HIV

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Dr Kerkhoff reported no relevant financial affiliations. (Updated June 27, 2023)

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Invited Review

CROI 2023: Summary of Basic Science Research in HIV

Mario Stevenson, PhD

University of Miami Miller School of Medicine, Florida

Abstract. *The 2023 Conference on Retroviruses and Opportunistic Infections (CROI) represented the first fully in-person conference since the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic began. CROI continues as the premier conference in which delegates can appraise themselves of almost every facet of HIV/AIDS research as well as emerging and re-emerging pathogens such as SARS-CoV-2 and mpox. The return to an in-person format is particularly important for early-stage investigators, who were faced with challenges of advancing their independent research careers during the SARS-CoV-2 pandemic. The personnel interactions and face-to-face meetings between junior investigators and their peers enable collaboration that is important in the academic development process. A very packed program showcased research advances in basic research, clinical, and epidemiology/public health endeavors around HIV and other pandemic viruses. Session presentation summaries, themed discussion sessions, and scientific workshops condense and assimilate specific areas of research that are particularly useful for delegates who want to see the state of research in areas that may be outside their specific areas of interest. The conference organizers drew on more than 1000 accepted abstracts to assemble a dynamic and engaging program that was appealing to infectious disease researchers worldwide.*

Keywords: CROI 2023, HIV, HIV-1, reservoirs, cure

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Virology

In his presentation in the Scott M. Hammer New Investigator workshop, Neil reviewed replication and immune escape strategies of HIV-1 and SARS-CoV-2. Much of the detailed understanding of HIV-1 infection has helped guide investigations into the inner workings of SARS-CoV-2. Both viruses are driven by competing selection pressures, such as those provided by host immune responses and target cell availability, and those pressures drive evolution in HIV-1 envelope glycoprotein and SARS-CoV-2 spike protein. Advances in imaging methods such as single-particle, cryo-electron microscopy (cryo-EM) have allowed visualization of the HIV-1 eceptor binding and conformational changes including trimer opening at remarkable levels of resolution (3.7 Å and 3.9 Å).

Work from several groups reveals the HIV envelope glycoprotein binding 3 CD4 proteins in rapid succession that induces conformational changes in the envelope, which exposes coreceptor binding sites. This CD4-induced conformational change in envelope opens new avenues for the development of therapeutics that prevent viral infection. One can see the impact of envelope evolution by following changes in the envelope sequence that occur in a single infected individual over the course of several years. Most of the changes lead to escape from neutralizing antibody and CD8+ T-cell responses. Some changes lead to increased CD4 binding affinity that typically can increase tropism for macrophages as well as changes in coreceptor use.

Similar forces appear at play in the evolution of the spike protein of SARS-CoV-2 and in the emergence of new variants of concern. Most of the changes over time erode the impact of neutralizing antibodies levied against the spike protein. This drives changes in spike protein conformation and alteration of viral biology. These themes were expanded on in presentations by Bieniasz (Abstract 18) and Hodcroft (Abstract 19). Neil reinforced the notion that changes occurring in the SARS-CoV-2 spike protein are not simply due to escape from host humoral immune responses, but change the

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basic biology of the spike protein itself. For example, the spike protein of some variants has a more open conformation, but that of the Omicron variant has a more closed conformation, which reduces dependence on the TMPRSS2 protease and is more dependent on endosomal entry than the original Wuhan strain of the virus. This also affects the sensitivity of the virus to antiviral membrane proteins such as interferon-induced transmembrane proteins (IFITMs) that have been shown to inhibit viral infection of diverse viruses such as Ebola virus, influenza A virus, and West Nile virus in different subcellular compartments, and guanylate binding proteins (GBPs) that mediate a broad spectrum of innate immune functions against viruses. Some of these changes are suspected to change the tropism of SARS-CoV-2 for epithelial cells in the upper respiratory tract that could potentially alter viral pathogenicity. Numerous lines of evidence suggest that HIV-1 has evolved mechanisms to limit pattern-recognition immune responses as well as to evade type I interferons that are triggered by this pattern-recognition response. This also appears to hold true for SARS-CoV-2 evolution outside of the SARS-CoV-2 spike protein (particularly in ORF6, ORF9, and N) in that some changes increase the level of expression of these viral accessory proteins that antagonize the innate antiviral forces of the cell (see also Abstract 108). These presentations collectively highlight the continuous evolution and adaptation of viruses that although driven primarily by escape from humoral and cell-mediated immune responses, also alter the biology of the virus to achieve greater fitness in the face of a hostile host environment.

For HIV, the molecular steps that immediately follow fusion of the viral membrane with the host cell membrane and precede integration of viral cDNA within host cell DNA remain the least well understood events in the viral replication cycle

For HIV, the molecular steps that immediately follow fusion of the viral membrane with the host cell membrane and precede integration of viral cDNA within host cell DNA remain the least well understood events in the viral replication cycle. This enigmatic phase of replication, collectively referred to as the preintegration steps of replication, is made of a number of successive events, some that distinguish the basic biology of lentiviruses from animal retroviruses. Once the virus has engaged receptor and coreceptor molecules, the core of the virion, which is made up of a capsid lattice containing genomic viral RNA and viral enzymes (eg, reverse transcriptase, integrase) that catalyze the cDNA synthesis and integration steps, respectively, is released into the cytoplasm of the target cell. Through an as yet poorly understood process, viral nucleic acids are reverse transcribed and transported to the nucleus of the cell, where the integrase enzyme promotes integration of viral cDNA with host cell DNA. This all occurs within a subviral capsid lattice, also referred to as the reverse transcription complex or preintegration complex.

Since the viral integrase needs to remain in association with nascent viral cDNA so that it can catalyze integration of that DNA, the subviral lattice has a mass that would otherwise preclude it from accessing the nuclear compartment of a cell that is not in mitosis. Myeloid cells do not divide, yet are permissive to HIV infection. Therefore, HIV-1 and other lentiviruses have evolved a mechanism that permits access of the preintegration complex to the nucleus. This is a central characteristic that underscores the general ability of lentiviruses to transduce nondividing cells and this property has been exploited for the derivation of lentivirus vectors for transduction of nondividing targets.

As discussed in several presentations (Abstracts 102, 104, 215, 216, and 217), there appear to be characteristics of the core that play several roles in the molecular events involved in cDNA synthesis and nucleic acid transport. The capsid protein that forms the core lattice mediates interactions with cellular factors that help guide the complex through the cytoplasm to promote further interactions that aid in docking the preintegration complex to the nuclear pore. The question remains whether the entire core/preintegration complex structure passes through the core as is, or undergoes some rearrangement that facilitates nuclear transport.

It now appears that the process of reverse transcription is triggered when the core docks at the nuclear pore, as opposed to the conventional view that reverse transcription is initiated prior to or immediately after fusion and proceeds concurrently with core transport through the cell cytoplasm. This likely serves as a viral defense mechanism that helps the virus avoid sensing by the host cell of viral cDNA by cytoplasmic DNA sensors. Mutations within the capsid can interfere with this avoidance mechanism and post-entry steps in viral replication in primary cells.

A number of immune proteins that target the capsid core as it traverses the cytoplasm, such as tripartite motif (TRIM) containing 5 (TRIM5) and TRIM containing 34 (TRIM34), have been identified. Mx2/MxB is an interferon-inducible guanosine triphosphate (GTP)ase that localizes to the nuclear pore complex and that has antiviral activity against a wide variety of viruses including lentiviruses, herpesviruses, and flaviviruses. As discussed in Abstract 104, the N-terminal domain of Mx2, which also harbors the nuclear localization signal mediating nuclear pore localization of Mx2, interacts with HIV-1 capsid and inhibits nuclear import of HIV-1 preintegration complexes. One presentation (Abstract 100) provided mechanistic insight into the antiviral activity of lenacapavir, the first-in-class HIV-1 capsid inhibitor. Although it prevents assembly of the viral capsid lattice during production of virions in the producer cell, it also interrupts preintegration events in viral replication. Lenacapavir binds to the capsid lattice after it enters the cytoplasm and interferes with interactions between the capsid lattice and host cell proteins (such as NUP153, Sec24C, and CPSF6) that are required for postentry functions of the capsid lattice. Intriguingly, capsid lattices stabilized by lenacapavir were able to access the nucleus but underwent abortive infection. In addition to their intrinsic value in management of HIV-1, antiviral agents such as lenacapavir can be used as research tools to help shed greater light on the enigmatic, preintegration events in HIV-1 replication.

Intriguingly, capsid lattices stabilized by lenacapavir were able to access the nucleus but underwent abortive infection

HIV-1 Persistence and Reservoir Studies

Analysis of the antiviral defenses levied against incoming viral genomes has provided provocative evidence that those defenses may play a key role in the establishment of viral latency. As highlighted in Abstract 37, antiviral defenses assault retroviruses not just as they traverse the cytoplasm, but also within the nucleus. It now appears that some of those antiviral defenses may aid in the establishment of latent HIV-1 infection.

During retroviral infection, some linear viral DNA molecules undergo end-to-end ligation and recombination to form 1- and 2-long terminal repeat (LTR) circles (viral episomes containing 1 or 2 copies of the LTR sequence). Some viruses, such as Epstein-Barr, can replicate from episomal DNA. However, viral gene expression from unintegrated DNA is extremely inefficient and viruses with mutations in integrase are also replication defective and accumulate repressive epigenetic marks, including trimethylation of lysine 9 on histone H3. Therefore, integration is a necessary step in the replication of HIV.

The processes that limit the expression of unintegrated viral DNA are not well understood. Using a genome-wide clustered regularly interspaced short palindromatic repeat (CRISPR)/Cas9 knockout screen, the Cullen laboratory identified the host SMC5/6 as orchestrating epigenetic silencing of unintegrated HIV-1 DNA.[1] SMC5/6 was shown to bind to small ubiquitin-like modifier (SUMO)ylate-unintegrated chromatinized HIV-1 DNA. Remarkably, when SMC5/6 expression was blocked, unintegrated DNA directed efficient transcription and integration defective mutants were replication competent. Surprisingly, blocking SMC5/6 expression also prevented establishment of latent HIV-1 infection in CD4+ T cells in vitro. The investigators propose that latent infection is a predetermined phenomenon that depends on SUMOylation of unintegrated DNA by the SMC5/6 complex.

This observation has important implications and suggests that HIV-1 latency is not merely a consequence of the activation state of the host cell or the region of chromatin that harbors the provirus, but rather, is an unfortunate side effect of a cellular innate immune response that originally was meant to silence foreign DNA. The current view is that latency is dictated by the activation state of the host cell. In activated cells, there are abundant cellular transcription factors that create an optimal environment for viral transcription, but in quiescent cells, these factors are rate limiting and therefore promote conditions for latency. Furthermore, studies suggest that proviruses integrated within nongenic regions of host cell chromatin are more likely to exist in a latent state and undergo selection over time, perhaps because they are less likely to be transcriptionally active and thus, less likely to be detected by host immune clearance forces. If indeed, latency is driven by epigenetic modification of viral cDNA prior to its integration, this could point to new strategies to limit the establishment of latency or to reverse latency.

The path to a cure for HIV-1 infection will be facilitated by a deeper understanding of the nature of the

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viral reservoir and characteristics of viral reservoir cells that might help inform on strategies to eliminate those reservoirs. As highlighted in Siliciano's presentation (Abstract 26) a concerted research effort has revealed some central features of the CD4+ T cell reservoir. The reservoir appears to be established early after infection and establishment of the latent reservoir is perhaps facilitated by initiation of antiretroviral therapy (ART). The reservoir comprises proviruses in memory CD4+ T cells that are expanded through the process of homeostatic proliferation. The intrinsic stability of the latent proviral reservoir in memory CD4+ T cells is a consequence of the longevity of memory CD4+ T cells. So far, host cell signatures that might distinguish a latently infected cell from an uninfected cell have not been identified. These basic characteristics of the memory CD4+ T-cell reservoir enforce the notion that elimination of this reservoir will be a formidable challenge. Researchers are now zeroing in on characteristics of the CD4+ T-cell reservoir at the single-cell level to better understand what might facilitate survival of the reservoir cell as well as host factors that might be differentially expressed in that cell and that could be exploited for therapeutic intervention.

Several excellent presentations highlighted and summarized advances in approaches to viral reservoir analysis that help to give a more detailed picture of the characteristics of individual reservoir cells (Abstracts 2, 3, 4, 135, and 142). A presentation by Roan (Abstract 2) overviewed the application of tools such as cytometry by time of flight (CyTOF) and single-cell RNA sequencing (scRNAseq) to the analysis of reservoir cells at the single-cell level. CyTOF uses mass cytometry to simultaneously quantitate various labeled proteins on the surface and the interior of individual cells. scRNAseq involves sequencing of cDNA libraries that were prepared from individual cells. Combined, these methods reveal the transcriptomic and proteomic content of individual cells in blood and in tissues. A limitation to these approaches is that they must look at many individual cells to find ones that are infected. Roan first discussed how these methods can reveal changes that occur in infected cells using tonsillar CD4+ T cells infected with an indicator virus. She illustrated how, on infection, CD4+ T cells exhibit characteristics not shown by uninfected cells—referred to as virus-induced remodeling. This information was then extrapolated to predict which subsets of T cells are most susceptible to infection. By comparing genes expressed in the infected cells, Roan was able to predict the nature of the original target cell, known as predicted precursor cells. This analysis was also applied to identify the HIV-susceptible cell subsets in the female reproductive tract.

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Prior studies from the same laboratory indicated that genital tract T cells are more susceptible to HIV infection than their counterparts in blood. Those cells were identified as memory CD4+ T cells. Naive CD4+ T cells were spared from HIV-1 infection. Within the memory T-cell population, T-effector memory and central T-resident memory cells were preferentially infected, and T-central memory cells were preferentially spared from infection. Roan was able to extend this by examining the protein signature of productively infected cells to identify the proteins that might be remodeled by infection. This analysis revealed that HIV infection downregulates T-cell receptor (TCR) signaling apparatus and promotes expression of factors such as surviving that promote T-cell survival and homing. This paints a more detailed picture of an infected cell and indicates that HIV-1 infection of the cell dampens the ability of the cell to respond to adaptive immune responses mediated through TCR signaling while simultaneously remodeling promoting their ability to survive and disseminate infection by inducing their migration to draining lymph nodes and retention in lymph node follicles.

Roan's laboratory has more recently extended the analysis to assess the glycan content of infected cells. This approach involves labeling of glycans with tagged lectins, an approach termed CyTOF-Lec. Through this modification, Roan was able to ask whether HIV-1 preferentially infects cells with specific glycan profiles and whether those profiles were remodeled after infection. HIV-1 was found to preferentially infect cells expressing high levels of fructose and sialic acid and further upregulated those glycans upon infection. Interestingly, the expression levels of sialic acid appeared to discriminate among a population of similar cells for susceptibility to infection. Sialic acid also plays a role in promoting evasion from natural killer (NK)-mediated recognition. Therefore,

HIV appears to select for cell subsets that might be able to provide refuge from NK-mediated killing.

These in vitro studies are now being extended to examine characteristics of reservoir cells from ART-suppressed individuals with HIV. The challenge to this analysis is that there is no marker that would aid in selecting the reservoir cells for analysis. Prior studies have circumvented this by ex vivo reactivation of reservoir cell, which in itself, would change the phenotype of the reservoir cell. Roan addressed this through the same approach used to predict the original transcriptomic and proteomic content of a cell infected in vitro. First, an atlas of all memory T-cell types is assembled. The information from a reactivated, infected cell is then matched against the atlas to reveal the identity of the original nonreactivated cell—the predicted phenotype. This analysis revealed the identity of several markers on memory T cells that could enable enrichment of reservoir cells from infected individuals followed by high-dimensional single-cell analysis that would not have been possible with unenriched cells. This allowed defining some of the characteristics of nonreactivated reservoir cells. One of those characteristics was the presence of viral RNA transcripts that have previously been used to define a component of the reservoir as the "expressed" viral reservoir. Prior studies have indicated that levels of cell-associated viral RNA in CD4+ T cells from individuals on ART can predict the time to viral rebound if ART is interrupted, and that the origin of rebound viremia includes cells with expanded proviruses that harbored viral transcripts prior to ART interruption.[2,3]

Abstract 106 discussed studies that attempted to assess the fraction of infected cells harboring viral RNA and whether there is a relationship between level of plasma viremia and the percentage of cells that harbor viral RNA. The frequencies of cells harboring viral RNA (unspliced, genomic RNA) were compared between untreated individuals with high levels of viremia, untreated individuals with low levels of viremia, and individuals on ART. Approximately 20% of infected cells from viremic and aviremic individuals were found to express viral RNA at any point in time. Noncontrollers were found to have a 20-fold higher frequency of viral RNA-containing cells than viremic controllers. There was also a direct correlation between number of viral RNA-containing cells and levels of plasma viremia. Surprisingly, however, the fraction of infected cells containing viral RNA was not associated with the level of plasma viremia. However, levels of viral RNA in single cells were correlated with plasma viremia and cells from viremic noncontrollers had higher levels of viral RNA per cell than those from viremic controllers.

Therefore, although viremic noncontrollers and controllers have similar frequencies of cells harboring viral RNA, viremic controllers have fewer infected cells, and as a consequence, fewer total cells expressing viral RNA. The investigators concluded that the natural control of HIV replication is not due to inhibition of proviral expression but to factors (such as cytotoxic T lymphocytes [CTLs], NK cells, and host factors) that limit viral replication from cells harboring expressed proviruses. Collectively, these analyses are providing deeper insight into reservoir characteristics. These approaches will hopefully address fundamental questions regarding the viral reservoir. For example, is the "expressed" viral reservoir distinct from the latent viral reservoir? What percentage of reservoir cells is transcriptionally active? If most reservoir cells are transcriptionally active, will they also express viral proteins, and if so, can this be exploited for viral reservoir clearance strategies?

Noncontrollers were found to have a 20-fold higher frequency of viral RNA-containing cells than viremic controllers

Betts (Abstract 3) also overviewed single-cell approaches for viral reservoir characterization at the DNA level. The approaches use the HIV provirus as the tag for the reservoir because, by definition, any reservoir cell must harbor a copy of the viral genome. In addition, using the provirus as a tag circumvents the need to reactivate the infection and as such, the original identity of the reservoir cell can be maintained.

One of the most recent approaches to reservoir analysis at the DNA level is FINDseq, developed by the Boritz research group, which uses a viral DNA probe to sort low numbers of reservoir cells in batches of approximately 100 cells that then underwent transcriptomic analysis. That approach revealed that viral DNA-containing cells exhibited transcriptomic signatures consistent with antideath and antiproliferative characteristics to the infected cell. This analysis was not optimal since it was not at single-cell resolution and was limited to the transcriptomic content of the cell.

Some of these issues were addressed in Abstract 1435, which described an approach called PHEPseq that combines viral DNA profiling with cell surface protein characterization. The approach targets viral DNA with

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primer-probe sets that identify intact versus defective (with deletions) provirus and allows identity of the cell to be determined from analysis of cell surface proteins. As such, the memory phenotype of the infected cell harboring intact, defective, and clonally expanded proviruses could be determined. Finally, Betts highlighted the utility of ASAPseq that detects viral DNA in genes in open chromatin and simultaneously uses oligo-tagged antibody to give information on the nature of the infected cell. The analysis showed that viral DNA was present in almost every CD4+ T-cell subset from individuals with HIV-1 infection on ART. As with data gleaned from other single-cell approaches, provirus-containing cells exhibited a trend toward expression of cell survival proteins. Taken together, these different approaches point toward a phenotype of a reservoir cell that has a propensity for survival and activation, a feature that favors reservoir longevity and renewal of viral replication when conditions for that replication are favorable.

Ho (Abstract 4) assessed approaches being adopted in her laboratory to answer basic questions on reservoir characteristics such as features of the reservoir cell that enable viral persistence in the face of viral cytopathicity and immune clearance. Ho uses an approach (ECCITE-seq) that allows analysis of transcriptomic profiles of infected cells together with their memory phenotype and their T-cell clone size. This method was applied to individuals at acute viremia and after 1 year of ART. Over this interval, the most clonally expanded CD4+ T cells exhibited a cytotoxic CD4+ cell phenotype expressing granzyme A and B and perforin. In addition, T-cell clones with the same antigen and immune program also contained expanded proviruses indicating that antigen responsiveness drives clonal proviral expansion. HIV-positive granzyme B-positive clones upregulated SERPINB9, which inhibits granzyme B. This supports a model in which the infected cell protects itself from self-inflicted granzyme B killing, again enforcing the notion that HIV infection remodels the infected cell for survival and self-protection. Although these characteristics appear representative of cells within the "expressed" viral reservoir, it is unclear whether those same characteristics are exhibited by reservoir cells that are not expressing HIV. Regardless, these studies provide a baseline for future studies to zero in on cell surface markers that could aid in the specific identification (and removal) of reservoir cells.

All cited abstracts appear in the virtual CROI 2023 Abstract eBook, available online at www.CROIconference.org

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Invited Review

CROI 2023: Tuberculosis and Infectious Complications in Persons With HIV

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Abstract. *Novel implementation strategies to increase uptake and adherence to tuberculosis (TB) preventive therapy hold promise for reducing TB incidence in persons with HIV in high-burden settings. In persons who develop drug-susceptible TB, progress to shorten TB treatment continues to be made with the introduction of new drugs and novel treatment strategies that could allow for treatment shortening to 2 months for most people. A global case series provided powerful evidence that mpox should be considered an HIV-related opportunistic infection given its severe manifestations and poor outcomes. Studies of TB and infectious complications in people with HIV presented at the 2023 Conference on Retroviruses and Opportunistic Infections (CROI) are summarized herein.*

Keywords: coinfection, CROI 2023, tuberculosis, TB, HIV, opportunistic infection, mpox, Kaposi sarcoma, anal cancer

Tuberculosis

Treatment in Adults

The development of anti-tuberculosis (TB) treatment regimens that are shorter and less toxic, but that remain highly effective, continues to be a priority for the field. For adults with or without HIV who have drug-susceptible TB (DS-TB), the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend a 4-month regimen of rifapentine (RPT), moxifloxacin (MOX), isoniazid (INH), and pyrazinamide (PZA) for 2 months followed by 2 months of RPT, MOX, and INH (RPT-MOX).[1,2]

At this year's Conference on Retroviruses and Opportunistic Infections (CROI), Paton and colleagues presented the results of the open-label, noninferiority, multicountry, randomized TRUNCATE-TB (Two-Month Regimens Using Novel Combinations to Augment Treatment Effectiveness for Drug-Sensitive Tuberculosis) trial that evaluated whether a novel, adaptive treatment strategy leveraging newer antimycobacterial agents could be used to go even further and reduce the treatment duration to as short as 8 weeks for many individuals (Abstract 113). Adults with confirmed rifampicin (RIF)-susceptible pulmonary TB were randomly assigned 1:1:1:1:1 to receive either the standard 6-month INH, RIF, PZA, and ethambutol (EMB) treatment regimen (2HRZE/4HR), or 1 of 4 different 5-drug regimens initially for 8 weeks, then extended to 12 weeks if there was evidence of ongoing symptoms and a positive sputum smear (at 8 weeks), then switched to complete the standard 6-month treatment if there were ongoing symptoms and a positive sputum smear again (at 12 weeks). The 4 different 5-drug investigational regimens were (1) high-dose (h)RIF + linezolid (LZD), (2) bedaquiline (BDQ) + LZD, (3) RPT + LZD, and (4) hRIF + clofazimine (CFZ), each in combination with INH/PZA/EMB (except RPT + LZD, which used levofloxacin instead of EMB). For pragmatic reasons (eg, challenges importing the drugs into trial countries), the trial stopped enrollment early for the RPT + LZD and hRIF + CFZ arms. The primary outcome was any unfavorable outcome (death, ongoing treatment, or active disease) up to 96 weeks, with a noninferiority margin of 12%. Notably, the trial initially excluded, but later allowed the inclusion of, persons with HIV and those with a very high mycobacterial burden (sputum smear 3+ positive or chest radiographic evidence of large, >4 cm cavitation). Among 674 participants (54% had chest cavitation present on chest X-ray [CXR], 0% persons with HIV), 92% of hRIF + LZD participants (n =

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184) completed treatment (78% and 11% completed 8 and 12 weeks of treatment, respectively), and 95% of BDQ + LZD participants (n = 189) completed treatment (86% and 7% completed 8 and 12 weeks of treatment, respectively), compared with 98% of participants (n = 181) receiving standard treatment. The percentages of participants with an unfavorable outcome at 96 weeks in the hRIF + LZD, BDQ + LZD, and standard treatment arms were 11.4% (21/184), 5.8% (11/189), and 3.9% (7/181), respectively. The BDQ + LZD regimen met the noninferiority margin (adjusted difference, 0.7%; 95% CI, −3.4 to 5.0) compared with standard treatment, and the hRIF + LZD regimen did not meet the noninferiority margin (adjusted difference, 7.4%; 95% CI, 1.7-13.2). The BDQ + LZD regimen also met the noninferiority margin in all predefined subgroup analyses, including among those with lung cavitation on CXR and with sputum smear grade 2+ or 3+ disease. The incidence of grades 3 or 4 adverse events was similar in the BDQ + LZD (11.1%) and hRIF + LZD (10.9%) treatment arms compared with standard treatment (13.8%). Thus, a novel, adaptive TB treatment approach using a BDQ + LZD-based, 5-drug regimen allowed treatment shortening to 8 weeks for 86% of persons with DS-TB and was noninferior to a traditional 6-month treatment regimen. This strategy could be further refined by understanding who is most likely to need standard 6-month therapy. In addition, this resource-intensive strategy could have less favorable outcomes under nontrial conditions; implementation research studies are needed to replicate these findings under real-world settings before this treatment strategy can be recommended and broadly scaled up.

In the TRUNCATE-TB trial, an adaptive strategy that aimed to reduce treatment duration for drug-susceptible TB using an initial 8-week regimen of bedaquilline, linezolid, isoniazid, pyrazinamide, and ethambutol followed by a treatment extension for individuals with persistent symptoms and smear-positive disease was noninferior to the standard 6-month TB treatment regimen

Current TB treatment regimens for DS-TB and drug-resistant TB (DR-TB) differ in the drugs used and the total duration of therapy. Newer TB drugs now make it possible to evaluate regimens that could be used for patients with DS-TB and DR-TB. In the SimpliciTB trial, Cevik and colleagues evaluated the BPaMZ regimen, consisting of BDQ, pretomanid (Pa), MOX, and PZA, for DS-TB (4 months) and DR-TB (6 months) (Abstract 109). DS-TB patients were randomly assigned 1:1 (open label) to receive the BPaMZ regimen for 4 months (4BPaMZ) or the standard 6-month regimen (2HRZE/4HR), and DR-TB patients received the BPaMZ regimen for 6 months (6BPaMZ). The primary study endpoint across the 3 arms was culture-negative disease at 8 weeks; however, a key secondary endpoint was relapse-free cure at 52 weeks (noninferiority margin, 12% for DS-TB participants). Overall, 455 participants were enrolled (19% were persons with HIV and 78% had lung cavitation present on CXR) including 150 and 153 DS-TB patients in the 4BPaMZ and 2HRZE/4HR arms, respectively, and 152 DR-TB patients in the 6BPaMZ arm; there was no DR-TB control arm. DS-TB and DR-TB participants receiving BPaMZ had a substantially higher likelihood of having culture-negative disease by week 8 (84.1% and 85.7%, respectively) than DS-TB participants receiving 2HRZE/4HR (47.3%); for DS-TB, this met the threshold of superiority. However, at 52 weeks, 16.7% (24/144) and 16.5% (22/133) of participants receiving 4BPaMZ and 6BPaMZ, respectively, had an unfavorable outcome compared with 6.9% (10/144) of participants receiving 2HRZE/4HR. Compared with DS-TB patients receiving 2HRZE/4HR, 4BPaMZ did not meet the threshold for noninferiority in the modified intention to treat analysis (mITT) (unadjusted risk difference, 9.7%; 95% CI, 2.4-17.1). Notably, study withdrawals due to adverse events accounted for 60.8% (28/46) of unfavorable outcomes in the BPaMZ arms and were predominantly due to elevated liver enzyme levels greater than 3 times the upper limit of normal (ULN); 7.5% (21/281) of all participants receiving a BPaMZ regimen had liver enzyme elevations greater than 8 times ULN. Thus, a novel 4-month regimen for DS-TB with BPaMZ had high mycobactericidal activity, but it was not noninferior to the standard 2HRZE/4HR regimen, in part due to concerningly high rates of hepatotoxicity.

In the context of treatment for HIV-associated TB, people with HIV receiving dolutegravir (DTG)-based antiretroviral therapy (ART) regimens are recommended to take DTG 50 mg twice daily to account for lower DTG levels due to RIF, a potent inducer of hepatic enzymes. Shah and colleagues evaluated HIV virologic suppression (≤1000 copies/mL) in adults with HIV-associated

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TB receiving DTG twice-daily ART during RIF-based TB treatment in public health programs in 6 resource-limited countries (Abstract 755). The 91 participants had a median CD4+ count of 120 cells/μL, and 87% had an initial HIV viral load above 1000 copies/mL. Of 73 participants with an HIV-1 RNA test result at the end of TB treatment, 68 of 69 (95%) had viral suppression, and 88% had below 50 copies/mL. None of the 4 nonsuppressed participants had emergent DTG resistance identified. The combined ART and TB regimens were well tolerated. Although numbers are limited, these data demonstrate the potential feasibility and effectiveness of DTG twice daily in people with HIV-associated TB in resource-limited settings.

Twice-daily DTG may be feasible during TB treatment, but it is more complex for programs to administer and for patients to take. Furthermore, based on prior data, it is not clear that the second DTG dose is needed. Griesel and colleagues undertook a phase IIb, randomized, double-blind, controlled trial to evaluate HIV virologic outcomes among persons with HIV with TB who received placebo (daily DTG, intervention) or supplemental twice-daily DTG (control) in addition to their normal ART regimen of tenofovir (TDF)/lamivudine (3TC)/DTG until 2 weeks after finishing RIF-based TB treatment (Abstract 110). Participants were followed up for 48 weeks to determine HIV virologic outcomes (RNA <50 copies/mL) and treatment-emergent DTG resistance. Among 108 enrolled participants, 81% were ART naive, the median CD4+ count was 184 cells/μL, and median HIV viral load was 5.2 $\log_{10}$ copies/mL. Characteristics were well matched between arms. HIV virologic suppression in both arms was similar during the follow-up period. At 24 weeks (the completion of TB treatment), 83% (95% CI, 70-92) were suppressed in both the intervention and control arms; however, at week 48 virologic suppression declined to 67% (95% CI, 53-90) and 69% (95% CI, 55-82) in the intervention and control arms, respectively. TDF concentrations in dried blood spots suggested that this decline largely reflected poorer adherence at week 48 than at week 24. None of the 19 participants with study-defined virologic failure had evidence of emergent DTG resistance. The most striking finding of this study was the low rates of viral suppression at 48 weeks in both arms due to poor ART adherence. Regarding the need for twice-daily DTG dosing in the presence of RIF, this small study provides some evidence that it may not be needed. Confirmation in a larger study would be required before once-daily DTG in the setting of TB can be widely recommended.

Treatment in Children

In the multicountry SHINE (Shorter Treatment for Minimal TB in Children) trial, for children with nonsevere DS-TB, a 4-month regimen (2 months RIF/INH/PZA with or without EMB, then 2 months RIF/INH) was noninferior to the standard 6-month regimen (2HRZE/4HR regimen for children with and without HIV). The composite endpoint was treatment failure, lost to follow-up, or death by 72 weeks.[3] This 4-month TB regimen is now recommended by the WHO as an option for treatment of nonsevere childhood and adolescent TB.[4] However, children with HIV may have poorer clinical TB treatment outcomes than HIV-negative children; therefore, Chabala and colleagues undertook a secondary analysis of the SHINE trial results to determine whether these outcomes differed according to HIV status (Abstract 824). Of 1204 enrolled participants, 11% (n = 127) were children with HIV, of which 54% (n = 68) were ART naive and the median CD4+ count was 719 cells/μL. Similar to previous studies, children with HIV were less likely to have microbiologically confirmed TB (6.3% vs 14.6%, respectively; $P < .001$) and more likely to have lymph node disease and to be underweight and anemic than children without HIV. Deaths were overall infrequent (2.6%; n = 31), but they were substantially higher among children with HIV (10.2%; n = 13) than among children without HIV (1.7%; n = 18; adjusted hazard ratio [aHR], 2.6; 95% CI, 1.2-5.8). The risks of hospitalization (adjusted odds ratio [aOR], 2.4; 95% CI, 1.3-4.6) and grades 3 or 4 adverse events (aOR, 4.4; 95% CI, 2.3-8.5) were also much higher among children with HIV. Among children with HIV with available virologic data, the proportion with a viral load below 1000 copies/mL was 45% and 61% at weeks 24 and 48, respectively. This study points to the need for new interventions beyond shortening TB treatment to reduce the unacceptably high morbidity and mortality in this population.

Although the indication for DTG-based ART therapy has recently been expanded to include children weighing 20 kg to 35 kg, there are limited data on the safety and potential efficacy of twice-daily DTG in the setting of TB treatment in children with HIV-associated TB. Therefore, Naidoo and colleagues undertook an open-label, nonrandomized, prospective pharmacokinetic (PK) study among 13 children with HIV receiving RIF-based TB therapy and DTG twice daily for HIV (Abstract 827). The median CD4+ count and HIV viral load among participants was 109 cells/μL and 2.5 $\log_{10}$ copies/mL, respectively. DTG PK parameters at steady state showed similar median trough concentrations (C_{Tau}) of 1.6 mg/L vs 1.5 mg/L and 24-hour area under the curve concentrations ($AUC_{0\text{-}24}$) of 33.6 h•mg/L vs 36.7 h•mg/L while receiving DTG twice-daily ART with RIF-based

TB therapy compared with once-daily DTG-based ART after stopping RIF-based TB therapy. All children had undetectable viral loads at weeks 12 and 24. Two participants had grade 3 adverse events (serum amylase level elevation), but no serious adverse events occurred. These preliminary data suggest that ART with twice-daily DTG during RIF-based TB therapy may be safe and well tolerated, but further data are needed to confirm these findings.

Isoniazid preventive therapy is associated with reduced TB incidence even in countries with high multidrug-resisant TB prevalence

Multidrug-Resistant TB (MDR-TB)

Linezolid is an important therapeutic option in the treatment of MDR-TB, but common and predictable toxic effects, namely, hematologic effects and peripheral neuropathy, often limit its longer-term use. Sutezolid is a novel agent closely related to linezolid that is proposed to have an improved safety profile. Heinrich and colleagues undertook a phase IIb, open-label, randomized, dose-ranging trial in which 75 participants (2 persons with HIV) with smear-positive DS-TB were randomly assigned to receive BDQ + delamanid + MOX and 1 of 5 sutezolid doses (range, 0 mg to 800 mg twice daily) for 12 weeks to evaluate safety and impact on decline in weekly sputum culture time to positivity (TTP) (Abstract 114). Sputum TTP increased over 12 weeks, suggesting declining mycobacterial burden across all 5 arms, but it did not significantly differ by arm. No episodes of neuropathy occurred, and 1 participant developed myelosuppression that was more likely attributable to nondrug-related causes. Although not directly compared, sutezolid appears to have an improved safety profile compared with linezolid, but it did not clearly improve antimycobacterial activity when given with a potent 3-drug regimen. Further study of the effectiveness and safety of sutezolid as part of a combination treatment for TB disease is warranted.

High-dose isoniazid (INH) may be beneficial as part of a multidrug treatment for MDR-TB, but it is not well known if it is effective against *Mycobacterium tuberculosis* (Mtb) strains containing mutations in the *katG* gene, which is a common cause of INH resistance. Gausi and colleagues undertook a phase IIA, open-label trial among patients with MDR-TB and *katG*-mediated INH resistance to evaluate early bactericidal activity (EBA) of 2 high INH monotherapy doses (Abstract 750). Twenty-one participants (4 persons with HIV) were randomly assigned 1:1 to receive either 15 mg/kg or 20 mg/kg INH daily for 7 days. Daily sputum was collected to determine TTP, and TTP was averaged over 7 days as a correlate of EBA. The initial average TTP was 143 hours, and the average TTP over the 7-day treatment period did not significantly change in either the 15 mg/kg group (1.8 hours/day; 95% CI, −1.7 to 7.3) or the 20 mg/kg group (2.4 hours/day; 95% CI, −1.3 to 5.5). These data suggest that high-dose INH likely has no benefit in the treatment of Mtb strains with *katG* mutations.

Prevention

Improving uptake of and adherence to TB preventive therapy (TPT) among persons with HIV is a key public health priority for reducing HIV-associated TB and related mortality. TPT is recommended by the WHO for all persons with HIV living in high TB burden settings following exclusion of active TB disease.[5] Shapiro and colleagues evaluated the effect of integrating isoniazid preventive therapy (IPT) initiation and continuation for persons with HIV into community-based ART-differentiated service delivery (DSD) models in KwaZulu-Natal, South Africa (Abstract 111). This represented a substudy of the DO ART (Delivery Optimization for Antiretroviral Therapy) study that previously showed that community-based ART improved virologic suppression compared with facility-based care.[6] Participants were randomly assigned 1:1:1 to receive ART and IPT via a facility-based care model (all services at health facility), a hybrid-care model (ART and IPT initiation at facility, monitoring and refills in the community via mobile van), or a community-care model (ART and IPT initiation, monitoring, and refills in the community via mobile van). All IPT refills were synchronized with quarterly ART refills. The relative risks (RRs) of initiating and continuing IPT (defined by IPT dispensed or by self report) in the hybrid-care and community-care model, relative to the facility-based care model, were determined. Of 1212 persons with HIV randomly assigned to start ART, 1039 started ART, of which 573 (55.1%) initiated IPT in the first year of study follow-up. Among initiators of ART (n = 1039), 19.7% of people in the facility-based care model, 48.0% in the hybrid-care model (RR, 2.4; 95% CI, 1.9-3.1), and 90.5% in the community-care model (RR, 4.6; 95% CI, 3.7-5.7) started IPT. Among those who initiated IPT (n = 573), IPT was "continued" by 48.5% of

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persons in the facility-based care model, 83.7% in the hybrid-care model (RR, 1.7; 95% CI, 1.3-2.4), and 89.4% in the community-care model (RR, 1.8; 95% CI, 1.4-2.4), respectively. These data suggest that a person-centered TPT delivery strategy integrated within community-based ART DSD models has the potential to improve TPT uptake and continuation among persons with HIV in high TB burden settings.

Hazardous alcohol use (HAU), which is common in people with HIV, is an important risk factor for TB disease and nonadherence to IPT. Chamie and colleagues undertook a 2x2 factorial, randomized, controlled trial among adult persons with HIV on stable ART in Uganda, who had evidence of latent TB infection (tuberculin skin test [TST] ≥5 mm) and HAU, to determine whether a novel strategy using conditional financial incentives could reduce alcohol use and improve IPT adherence during the 6-month daily course (Abstract 112). Participants were randomly assigned 1:1:1:1 to 1 of 4 arms: arm 1, no incentives, control; arm 2, incentives contingent on no recent alcohol use (determined using urine-based point-of-care [POC] ethyl glucuronide test, a biomarker of recent alcohol use); arm 3, incentives contingent on IPT adherence (determined using urine-based POC IsoScreen assay, a biomarker of recent INH use); or arm 4, incentives for satisfying either no recent alcohol use or IPT adherence, or both. The intervention arms (arms 2, 3, and 4) used an escalating financial incentive structure, such that for each consecutive month of negative POC urine test results, participants would be awarded more scratch cards with differing values (range $5 to $50 USD); the number awarded would reset if POC tests revealed recent alcohol use or lack of recent IPT. Of 680 persons with HIV, 69.1% were male and 90.2% were virologically suppressed (HIV RNA <40 copies/mL). Baseline characteristics were well balanced across arms. Overall, participants receiving financial incentives were more likely to have no hazardous alcohol use (measured by self-report and blood biomarker) than those who did not receive financial incentives (17.6% vs 9.9%, respectively; P = .003). However, there was no difference in IPT adherence (measured by electronic bottle cap openings) between incentivized and nonincentivized arms (72.8% vs 72.9%, respectively; P = .944). This study shows that contingent, escalating financial incentives reduced heavy alcohol use among persons with HIV receiving IPT, but it did not improve adherence to a 6-month, daily IPT regimen.

HAU may serve as a key barrier to starting IPT in persons with HIV, as it is recommended that persons with HAU do not receive IPT, given concerns of hepatotoxicity. Hahn and colleagues undertook a single-arm trial in Uganda among persons with HIV on stable ART with a positive TST (≥5 mm) and normal liver transaminase levels (aspartate aminotransferase [AST] and alanine transaminase [ALT] levels ≤2x ULN), and who either reported recent alcohol use (last 3 months, n = 200) or no recent alcohol use (last 1 year, n = 101) to evaluate the frequency of severe hepatoxicity during IPT (Abstract 743). Monthly visits until 1 month following IPT completion were conducted to assess INH refills, to measure transaminase levels, and to monitor symptoms. Of 301 participants enrolled, 92.1% were virologically suppressed. Twenty-five participants (8.3%) experienced a grade 3 or higher INH-related toxicity (defined as AST or ALT ≥5x ULN); 12 of 200 (6.0%; 95% CI, 3.1-10.2) occurred in those reporting recent alcohol use and 13 of 101 (12.9%; 95% CI, 7.0-21.0) were in those reporting no recent alcohol use. There was a trend toward grade 2 toxicities (AST or ALT, 2-5x the ULN) being more common among those reporting recent alcohol use (25.0%; 95% CI, 19.0-31.8) than those reporting no alcohol use in the past year (14.8%; 95% CI, 8.1-23.9). Multivariable analyses demonstrated that biomarker-confirmed degree of recent alcohol use (using phosphatidylethanol) was not associated with grade 3 or higher hepatotoxicity. However, high and very high recent alcohol use was independently associated with grade 2 hepatoxicity (aOR, 3.6; 95% CI, 1.4-8.9). These data suggest that unless baseline transaminase levels are greater than 2x the ULN, IPT in persons with HIV reporting alcohol use is unlikely to be associated with severe hepatoxicity, and thus among such patients this important preventive tool should not be deferred.

It is not well known whether IPT can effectively reduce TB incidence among persons with HIV in settings where there is a high prevalence of MDR-TB, which by definition includes resistance to INH. Sodeke and colleagues undertook a retrospective study based in Ukraine, a country with a high MDR-TB burden (31% of national notifications), from 2018 to 2022 using national electronic medical records, to evaluate TB incidence among persons with HIV who had received IPT (Abstract 758). Overall, 128,314 persons with HIV with complete data on IPT and TB diagnosis were included; 66.2% (n = 84,901) had received no IPT (defined as <28 days of IPT), 8.4% (n = 10,787) had received partial IPT (defined as 28-146 days of IPT), and 25.4% (n = 32,626) completed IPT (defined as >146 days of IPT). The adjusted rates of TB incidence were 2.1, 3.9, and 9.8 cases per 100 person-years for those with complete IPT, partial IPT (incidence rate ratio [IRR] compared with complete IPT, 1.9), and no IPT (IRR = 4.7), respectively. These rates were similar across the 4 study observation years. The proportion of participants with

incident MDR-TB diagnosed did not significantly differ across IPT groups (33.9% no IPT, 29.8% partial IPT, and 33.1% complete IPT). These population-level data offer compelling evidence that IPT can effectively reduce TB incidence among persons with HIV in high MDR-TB burden settings.

Diagnosis and Case Finding

The Xpert MTB/RIF Ultra assay using sputum is recommended by the WHO as a first-line assay for the diagnosis of pulmonary TB, but its performance on urine for extrapulmonary/disseminated disease is poorly characterized. Stead and colleagues undertook a cross-sectional study in South Africa to evaluate the diagnostic yield for TB of the bedside urine lipoarabinomannan (LF-LAM) POC test and Xpert on centrifuged urine (urine Ultra), alone and in combination, in consecutively enrolled persons with HIV requiring hospitalization with presumptive TB (Abstract 761). Among 238 participants (median CD4+ count, 76 cells/μL), 62 (26.1%) had confirmed TB diagnoses and 92 (38.7%) had definite or probable TB diagnoses. Overall, urine LF-LAM had a sensitivity and specificity of 55% and 90%, respectively, and for urine Ultra it was 70% and 100%, respectively. For definite TB, the yield of sputum Ultra was 34% (n = 21), urine LF-LAM was 45% (n = 28), urine Ultra was 68% (n = 42), and urine LF-LAM + Ultra was 73% (n = 45). For definite or probable TB, the yield of sputum Ultra was 23% (n = 21), urine LF-LAM 39% (n = 36), urine Ultra was 57% (n = 52), and urine LF-LAM + Ultra was 64% (n = 59). Urine Ultra also detected 5 cases of RIF-resistant TB. These data showed that urine Ultra had improved accuracy for TB compared with urine LF-LAM, and that both urine-based tests had improved yield compared with sputum Ultra, given the ease of urine collection. When available in high TB burden settings, urine Ultra testing should be used to improve TB detection among hospitalized persons with HIV.

HIV testing among close household contacts of persons with newly diagnosed TB represents an important opportunity for knowing one's status and linking to care, but in some settings up to 50% of household contacts identified through household contact tracing decline HIV testing. Armstrong-Hough and colleagues undertook a cluster (household-level) randomized trial to evaluate the efficacy of a brief social behavioral "norming" strategy to increase acceptance and uptake of HIV testing among contacts (≥15 years old) without known HIV, during TB household contact tracing in Kampala, Uganda (Abstract 1050). The brief norming intervention had several components including the following: (1) guided selection of the first tester in the household most likely to accept testing; (2) use of a standardized, encouraging script by community health care workers (CHWs); (3) HIV test offered using opt-out framing; (4) optional invitation for the first tester to share the decision to test (but not results) with other household members; and (5) masking of household members' decision not to test.[7] Overall, in the intervention arm there were 99 index TB patient households with 328 total contacts, and in the standard of care arm there were 86 index TB patient households with 224 total contacts. Uptake of HIV testing was higher in the intervention arm (98%) than the control arm (92%) (difference, +6%; 95% CI, 2-10; P=.004). CHWs reported that the norming strategy required similar time to the standard HIV testing strategy. This study demonstrated that a brief intervention to normalize HIV testing was feasible and could provide a small increase in HIV testing uptake among TB household contacts.

Opportunistic Infections

For cryptococcal meningitis, flucytosine (5FC) acts as an important component of the induction phase for some regimens by reducing the time to cerebrospinal fluid sterilization. However, the requirement for dosing 4 times a day is challenging, especially in resource-limited settings. Krantz and colleagues undertook a phase I, open-label, randomized, single-dose, 4-period, crossover study among 37 health participants to evaluate the safety and PKs of 3 different prototypes (B, C, and D) of sustained-release (SR) 5FC prototype pellets given once (1 x 3000 mg at 0 hours) compared with 5FC immediate release (IR) (A) (3 x 500 mg given twice at 0 and 6 hours) (Abstract 503). No serious or adverse events were identified during any of the 4 treatment phases. The $AUC_{0\text{-}t}$ (μg•h/mL) of the 3 prototypes was 179 for prototype B, 228 for prototype C, and 258 for prototype D, compared with 472 for prototype A, the reference IR formulation. Physiologically based pharmacokinetic (PBPK) modeling suggested a double dose of prototype D could achieve similar 5FC concentrations to 5FC IR under fasting conditions. These preliminary data suggest 5FC SR pellets could be available in the future to simplify CM treatment, and the double dose of prototype D will be evaluated in further phase I and II studies.

Pneumocystis jirovecii pneumonia (PJP) still occurs in the modern ART era. Epling and colleagues undertook a retrospective, US-based cohort study of 81 persons with HIV with a history of PJP and compared characteristics and outcomes up to 96 weeks in persons with HIV without prior PJP, but with a CD4+ count nadir below 100

cells/μL (Abstract 747). Of 81 participants with a history of PJP, 64 (79.0%) had their PJP diagnosed within 100 days of ART initiation. The median baseline CD4+ count in the PJP group was 14 cells/μL, compared with 24 cells/μL in the non-PJP group ($P < .001$). Compared with persons with HIV with no history of PJP, persons with HIV and a history of PJP had similar 96-week CD4+ cell counts and plasma HIV viral loads. Cytomegalovirus end-organ disease was associated with receipt of steroids for PJP (OR, 2.3; 95% CI, 1.0-5.0). Persistent chest computed tomography (CT) changes more than 1 year later were more common among persons with HIV with prior PJP but were relatively rare overall; 11% had bronchiectasis and 11% had subpleural cysts. Although no deaths were directly attributable to PJP, persons with a history of severe PJP had a substantially higher risk of mortality through 96 weeks (hazard ratio [HR], 6.2; $P = .046$) and notably most deaths occurred more than 24 weeks after ART initiation. PJP remains an important HIV-related opportunistic infection, and these data show that in the era of modern ART, persons with HIV presenting with severe disease remain at increased risk for poor outcomes and should be closely monitored after recovery from their acute illness.

Kaposi sarcoma (KS) also still occurs in the modern ART era. Martin and colleagues undertook a cohort study of adult persons with HIV with newly diagnosed, pathologically confirmed KS in Kenya and Uganda between October 2021 and August 2022, to determine their extent of disease at baseline and vital status over time (Abstract 150). Among 180 persons with HIV, the median CD4+ count was 197 cells/μL (26% had a CD4+ count of 50 cells/μL or below), and 46% had an HIV viral load of 40 copies/mL or below. Overall, 86% had evidence of advanced KS at the time of diagnosis, with a median of 7 anatomic sites of involvement with KS lesions per participant. There were 56 participants who died during the follow-up period, and the cumulative incidence of death at 2, 6, and 8 months following KS diagnosis was 24%, 33%, and 38%, respectively. These concerning contemporary data from East Africa demonstrate that KS is still being diagnosed at late stages with advanced disease and that short-term survival is poor. Innovations are needed for earlier detection and treatment of HIV and KS in sub-Saharan Africa.

Talaromycosis, which is caused by the dimorphic fungus *Talaromyces marneffei* (Tm), is a leading cause of death in persons with HIV in Southeast Asia, in part due to challenges with timely diagnosis that include a reliance on blood cultures, which may take up to 4 weeks to grow. Nguyen and colleagues undertook a prospective diagnostic accuracy study of a novel enzyme immunoassay (EIA), which detects a Tm-specific cell-wall antigen (Mp1p) in urine or blood, among hospitalized adults with advanced HIV disease (CD4+ count <100 cells/μL or WHO stage III/IV disease) in Vietnam (Abstract 765). All participants had blood cultures performed, as well as microscopic evaluation and culture of other specimens as clinically indicated. They were followed up for 6 months for the development of culture-confirmed talaromycosis to inform a composite reference standard. Among 662 participants, the overall prevalence of talaromycosis was 16.8% (95% CI, 14.0-19.9; n = 111 diagnoses). The sensitivity and specificity of the Mp1p assay on serum and urine samples were 82.0% and 96.0%, and 81.1% and 98.0%, respectively; both had greater sensitivity than conventional blood culture for the diagnosis of talaromycosis (66.7%). In this study, in which a high prevalence of talaromycosis was identified, the Mp1p assay demonstrated favorable diagnostic accuracy, especially compared with current culture-based approaches, and if further developed may be a useful first-line test among hospitalized persons with HIV in settings endemic for talaromycosis.

Phase I data suggest sustained-release flucytosine pellets could one day be used to simplify cryptococcal meningitis treatment

Human papillomavirus and anal cancer rates are higher in persons with HIV. At last year's CROI, Palefsky and colleagues shared the practice-changing results of the ANCHOR (Treatment in Preventing Anal Cancer in Patients With HIV and Anal High-Grade Lesions) study, which showed that early treatment of anal high-grade squamous intraepithelial lesions (HSILs) reduced the risk of anal cancer in persons with HIV by 57%.[8] One crucial gap in knowledge following these results is how to optimize screening for identifying HSIL, especially as the current standard screening approach. Anal cytology has poor specificity (46%-65%), which results in many patients requiring high-resolution anoscopy (HRA). Serrano-Villar and colleagues recruited 213 persons with HIV undergoing HSIL screening with HRA, who had anal biopsies performed to confirm HSIL, in order to identify anal microbiota predictive of HSIL (Abstract 148). The median CD4+ count of participants was 704 cells/μL, and 94%

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were men who have sex with men. There were several proteins overexpressed by bacteria in the anal microbiome of participants with HSIL, and they frequently converged in the production (and increased levels) of succinyl-CoA and cobalamin. Compared with anal cytology screening, the combination of succinyl-CoA and cobalamin had improved accuracy for HSIL, where the sensitivity was 96.6% versus 91.2%, respectively, and specificity was 81.8% versus 34.1%, respectively. Further, succinyl-CoA and cobalamin screening would have reclassified 49 of 61 (82%) false-positive cytology-based HSILs diagnosed to true negative diagnoses. Succinyl-CoA and cobalamin are promising biomarkers for improving HSIL screening in persons with HIV and should undergo further development and validation.

Mpox is an opportunistic infection in persons with advanced HIV defined by numerous severe complications and high mortality risk

Mpox—A New Opportunistic Infection

During the outbreak of mpox across 110 countries in 2022 and 2023, up to 50% of the approximately 85,000 cases were among persons with HIV. Most people had CD4+ counts above 500 cells/µL, were virologically suppressed on ART, and had similar clinical features and outcomes to persons without HIV. To address limitations in knowledge regarding mpox outcomes in persons with advanced HIV disease,[9] Orkin and colleagues undertook a global case series (19 countries) of persons with HIV with CD4+ counts below 350 cells/µL and polymerase chain reaction (PCR)-confirmed mpox to characterize the natural history of disease (Abstract 173). Of 382 persons, 96% were cisgender men, 8.6% had a new HIV diagnosis, the median CD4+ count was 211 cells/µL (22.3% had a CD4+ counts below 100 cells/µL), 50.5% had an HIV viral load below 50 copies/mL, and 8.4% had a concurrent opportunistic infection. People with CD4+ counts below 100 cells/µL were substantially more likely than those with a CD4+ count above 300 cells/µL to have severe complications with new mpox. These clinical manifestations, some of which have not been previously reported, included large, painful, coalescing necrotizing skin conditions (54.1% vs 6.7% for lower vs higher CD4+ counts, respectively); lung involvement (29.4% vs 0%, respectively); ocular complications (15.3% vs 1.3%, respectively); anorectal complications (52.9% vs 28.0%, respectively); genitourinary complications (34.1% vs 9.3%, respectively); and secondary bacterial infections (43.5% vs 9.3%, respectively). In the overall cohort, 28.0% of patients were hospitalized and 7.1% died (all 27 deaths occurred in persons with CD4+ counts below 200 cells/µL); among those with CD4+ counts below 100 cells/µL, 62.4% were hospitalized and 27.1% died, compared with 16.0% and 0%, respectively, among those with CD4+ counts above 300 cells/µL. Immune reconstitution inflammatory syndrome was suspected in up 24.7% of patients (n = 21/85) started or restarted on ART, but due to the observational nature of the data, it is not possible to infer recommendations on ART start time. These data demonstrate that mpox behaves as an opportunistic infection in persons with advanced HIV, and it is a distinct clinical syndrome marked by various severe complications and a high risk of mortality.

All abstracts cited in the text appear in the CROI 2023 Abstract eBook, available online at www.CROIconference.org

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review activities, eg, data monitoring boards, statistical analysis, or endpoint adjudication committees with Gilead Sciences, Inc. (Updated June 30, 2023) Reviewers 2 and 3 reported no relevant financial relationships with ineligible companies. (Updated April 23, 2023)

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Invited Review

CROI 2023: Metabolic and Other Complications of HIV Infection

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Abstract. *Comorbid conditions have major impacts on the health, quality of life, and survival of people with HIV, particularly as they age. The 2023 Conference on Retroviruses and Opportunistic Infections (CROI) featured excellent science related to specific comorbidities, such as cardiovascular disease (CVD), cancer, and obesity. Studies investigating factors that may contribute to CVD, such as mental health disorders, antiretroviral therapies, and activation of hormonal pathways, were featured prominently. Other studies sought to understand the epidemiology of non–AIDS-defining cancers in people with HIV. As at previous CROI conferences, weight gain attributable to antiretroviral therapies was a major theme, and several abstracts focused on the important question of whether weight decreases after discontinuation of antiretroviral therapy (ART) regimens associated with weight gain. This review focuses on abstracts presented at CROI 2023 in these areas, highlighting those with the most clinical impact.*

Keywords: CROI 2023, HIV, metabolic complications, comorbidities, antiretroviral therapy

Cardiovascular Disease in HIV

Anxiety and Depression and Myocardial Infarction

In addition to traditional cardiovascular disease (CVD) risk factors (smoking, hypertension, diabetes mellitus, and dyslipidemia), common mental health disorders may also contribute to CVD risk in people with HIV. In an analysis of more than 33,000 people participating in 7 clinical cohorts of NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design) in the US, Hyle and colleagues investigated the associations of depression and anxiety with myocardial infarction (MI) (Abstract 145). This study examined 2 MI outcomes: type 1 MI, which is related to rupture of an atherosclerotic plaque and myocardial damage to areas distal to the arterial occlusion; and type 2 MI, which occurs when oxygen demand outstrips oxygen supply and is observed with secondary conditions such as substance use disorder, arrhythmias, and heart failure.

Among people with HIV, almost half (49.4%) had a history of depression or anxiety. During the follow-up period between 1998 and 2017, a total of 869 MIs were observed, of which 495 (57%) were type 1 and 374 (43%) were type 2. For type 1 MIs, the presence of depression was associated with a 23% increased risk of MI (adjusted hazard ratio [aHR], 1.26; 95% CI, 1.02-1.49), whereas anxiety was not associated with increased risk (aHR, 0.92; 95% CI, 0.74-1.16). Other factors associated with type 1 MIs were male sex, older age, tobacco smoking, hypertension, dyslipidemia, diabetes, renal disease, and protease inhibitor use. For type 2 MIs, anxiety increased the risk by 42% (aHR, 1.42; 95% CI, 1.10-1.83), with a similar trend for depression (aHR, 1.20; 95% CI, 0.96-1.51). In addition to traditional CV risk factors, type 2 MI was also associated with cocaine use and detectable levels of HIV RNA.

These data suggest independent effects of these mental health conditions on CVD risk and lead to the question of whether appropriate linkage to care and treatment of these conditions will improve CVD risk in the future. Another important consideration based on these data is whether persons with either anxiety, depression, or both may potentially benefit from more aggressive CVD risk reduction.

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Blocking the Renin-Angiotensin-Aldosterone System

Activation of the renin-angiotensin-aldosterone system (RAAS) has been described in people with HIV and may play an important role in the pathogenesis of CVD (see Srinivasa for review).[1] As a final step in this pathway, aldosterone binds to the mineralocorticoid receptor in the kidney, which regulates sodium balance and blood volume; also, excessive RAAS activation in the heart leads to vascular dysfunction as well as myocardial injury and fibrosis. Mineralocorticoid receptor activation in macrophages and lymphocytes increases the elaboration of proinflammatory cytokines. Attenuation of RAAS activation through mineralocorticoid receptor antagonism may be an important strategy to decrease the CVD burden in people with HIV.

Activation of the renin-angiotensin-aldosterone system (RAAS) has been described in people with HIV and may play an important role in the pathogenesis of CVD

Srinivasa and colleagues investigated the effect of RAAS antagonism on cardiovascular function in people with HIV, using eplerenone, a US Food and Drug Administration (FDA)-approved mineralocorticoid receptor antagonist (Abstract 144). In a 12-month, placebo-controlled, randomized clinical trial of 40 antiretroviral therapy (ART)-treated people with HIV with central adiposity but without established CVD, more participants in the eplerenone group exhibited improved coronary flow reserve (CFR), as measured using coronary positron emission tomography, than those in the placebo arm. Moreover, among participants with impaired baseline CFR, those receiving eplerenone showed improvement in CFR compared with participants in the placebo group ($P = .04$). Eplerenone treatment was also associated with improvements in left ventricular end-diastolic volume ($P = .03$) and stress myocardial blood flow ($P = .03$). In addition to its cardiovascular effects, eplerenone treatment was associated with higher CD4+ T-cell count ($P = .02$) and a trend toward lower levels of high-sensitivity interleukin-6 (IL-6) ($P = .07$).

In summary, in this small, randomized trial, eplerenone treatment led to favorable changes in measurements of subclinical cardiovascular function as well as improved CD4+ T-cell count. Larger studies are needed to better understand the potential clinical benefit of eplerenone in people with HIV.

Are Integrase rand Transfer Inhibitors Associated With Heart Disease Events?

In the RESPOND (International Cohort Consortium of Infectious Disease) study, a large, multicenter cohort of people with HIV in Europe and Australia, exposure to integrase strand transfer inhibitors (InSTIs) was associated with an increased risk of CVD events over the first 2 years.[2] Surial and colleagues examined this important question in the Swiss HIV Cohort study; they investigated individuals with HIV who were treatment-naive before starting either InSTI or non–InSTI-containing ART (Abstract 149). The endpoint was the first cardiovascular event, defined as MI, stroke, or arterial intervention. Baseline characteristic differences between the InSTI and other ART groups were as follows: the InSTI group had fewer women and people of African origin as well as higher median CD4+ cell count nadir among participants. In adjusted analyses, the risk differences for CVD between the 2 groups were not statistically significant at 1 year (−0.02%; 95% CI, −0.32 to 0.21%), 2 years (−0.17%; 95% CI, −0.65 to 0.10%), or 5 years (−0.38%; 95% CI, −1.29 to 0.52).

In contrast to findings from the RESPOND study, this investigation did not confirm an association between InSTI exposure and CVD events. Additional data are needed to better understand whether InSTI exposure truly increases CVD risk.

Cancer Epidemiology in HIV

Cancer is a leading cause of death among people with HIV. Numerous important questions remain regarding cancer risk in people with HIV and the extent to which incidence differs from that in people without HIV, particularly for non–AIDS-defining cancers (NADCs), namely, breast, colon, head and neck, kidney, laryngeal, liver, lung, oropharyngeal, pancreatic, prostate, and anal cancers, as well as leukemia and Hodgkin's lymphoma.

Rudolf and colleagues examined incident cancers in Medicaid beneficiaries enrolled in 14 US states from 2001 to 2015; enrollees included more than 43 million people without HIV and 181,000 people with HIV (Abstract 155). For men and women, various NADCs, including leukemia and lung, head and neck, liver,

oropharyngeal, laryngeal, and anal cancers, were more common in people with HIV than in the general population. For colon cancer, the incidence was higher at younger ages in people with HIV but higher at older ages in people without HIV. For breast cancer, risk for early disease was similar for women whether with or without HIV, but after age 42, women with HIV had a lower risk than women without HIV. For prostate cancer, before age 50, men with HIV had a higher risk than men without HIV; however, after that age, the risk was higher among men without HIV.

The mechanisms underlying these risk differences by HIV serostatus as well as the interactions between age and HIV serostatus, as observed with some cancers, deserve further inquiry. Understanding how the differences in cancer incidence by HIV serostatus might impact screening practices is also important.

Are Cancer Outcomes Any Different in HIV?

Rava and colleagues compared mortality from NADCs in participants in the Spanish AIDS Research Network (CoRIS) cohort versus mortality in the general Spanish population between 2004 and 2020 (Abstract 871). Of the cancers examined, lung and liver cancers had the highest incidence in CoRIS participants. When NADCs were grouped together, mortality rates were higher in people with HIV than in the general population at younger ages (<60 years old), with the greatest difference by HIV serostatus observed in persons less than 40 years old. Factors associated with NADC mortality were viral hepatitis infection, smoking, and lower CD4+ count. It is unclear which cancers accounted for the differences in mortality by HIV serostatus and what factors may account for this difference (eg, stage at cancer diagnosis, type of or adherence to cancer treatments, and social determinants of health).

Does Obesity Contribute to Inflammation in HIV?

Overweight and obese states are steadily increasing in people with HIV, in parallel with increases in diseases associated with elevated weight. Because obesity is considered a proinflammatory state and chronic inflammation is thought to contribute to comorbidities in people with HIV, the relationship between inflammation and elevated body mass index (BMI) is of particular interest. Gelpi and colleagues studied the association between BMI and inflammation in people with HIV and in individuals without HIV in the Copenhagen Comorbidity in HIV Infection study (Abstract 253).

In an adjusted analysis, those with HIV with either normal or below-normal weight had greater levels of interleukin (IL)-6 (adjusted odds ratio [aOR], 5.82; 95% CI, 1.69–20.05) and interferon (IFN)-γ (aOR, 3.41; 95% CI, 1.01–11.46) than individuals without HIV. In contrast, greater levels of IL-6 and IFN-γ were not observed in people with HIV who were overweight or obese than in people without HIV with similar BMIs. Among participants with normal or below-normal weight, associations were observed in individuals with HIV between greater IL-6 levels and waist-to-hip ratio, age, and smoking, but not in participants without HIV.

This study suggests that people with HIV who have normal or below-normal BMI may have unique factors that predispose them to greater inflammation, such as the distribution of adipose tissue

This study suggests that people with HIV who have normal or below-normal BMI may have unique factors that predispose them to greater inflammation, such as the distribution of adipose tissue. It also suggests that inflammation in people with HIV who are obese is not accentuated compared with persons without HIV who are obese.

Antiretroviral Therapy–Related Weight Gain: Is It Reversible?

InSTIs and tenofovir alafenamide (TAF) each have been associated with weight gain in people with HIV. An important clinical question is whether discontinuation of these drugs will lead to decreased weight and improved metabolic health. In SOLAR, a 12-month, phase IIIb noninferiority efficacy study, Tan and colleagues studied the effect of continuing on a treatment regimen that includes bictegravir (BIC) and TAF (BIC/emtricitabine [FTC]/TAF) versus switching from BIC/FTC/TAF to cabotegravir (CAB) and rilpivirine (RPV) (given as a long-acting

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[LA] injection every 2 months) (Abstract 146). In the CAB + RPV LA arm, 38% of participants had overweight state and 21% had obesity, and in the BIC/FTC/TAF arm, 34% of participants had overweight state and 23% had obesity. As such, more than 50% of participants in each arm had overweight state or obesity.

In ART initiation and switch studies, tenofovir alafenamide is associated with more weight gain than tenofovir disoproxil

The change in weight from baseline to the end of the study was similar in the 2 arms. The median (interquartile range [IQR]) weight change from baseline to the end of the study in the CAB + RPV LA arm was −0.40 kg (−2.95, 2.10) and in the BIC/FTC/TAF arm was 0.05 kg (−2.30, 1.95). Similarly, changes from baseline in waist circumference, waist-to-hip ratio, and the proportion of individuals with insulin resistance (as measured by a homeostatic model assessment for insulin resistance [HOMA-IR] ≥2) were similar between the 2 arms. This study indicates that switching patients to CAB + RPV LA from BIC/FTC/TAF (ie, removing BIC and TAF) is unlikely to lead to decreased weight and improved metabolic health.

Does Switching From TAF to TDF Decrease Weight?

In ART initiation and switch studies, TAF is associated with more weight gain than tenofovir disoproxil (TDF). These switch studies have examined the effect of switching from TDF to TAF, rather than switching from TAF to TDF. This is an important clinical issue for people who have gained weight while receiving TAF and may be considering switching to TDF to better manage their weight.

Bosch and colleagues investigated whether weight gain after initial ART containing TAF could subsequently be reversed (Abstract 671). Participants in the ADVANCE (Dolutegravir Plus Two Different Prodrugs of Tenofovir to Treat HIV) trial in South Africa were randomly assigned to 1 of 3 arms: TAF/FTC/dolutegravir (DTG), TDF/FTC/DTG, or TDF/FTC/EFV for 192 weeks, after which participants were given TDF/lamivudine (3TC)/DTG in an open-label arm as part of the CHARACTERISE (a Cross-sectional, Observational Study to Characterise the Transition to Dolutegravir-Based Regimens in South Africa in Terms of the Emergence of Obesity, Viral Re-suppression, and Integration Into Routine Programme Care) trial, as this combination is the standard of care in South Africa. Those who received TAF/FTC/DTG in ADVANCE and subsequently switched to TDF/3TC/DTG in CHARACTERISE had weight loss of 1.2 kg (P = .01) and decreased values for hemoglobin A1c (−0.10 mmol/L; P = .008), fasting glucose (−0.20 mmol/L; P = .001), and low-density lipoprotein cholesterol (−0.32 mmol/L; P = .001) levels. The effects appeared to be driven by women who lost approximately 4 kg over the 48 weeks after the switch, which equaled approximately 40% of the weight gained during the 192 weeks of ADVANCE. It is unclear whether the weight loss would have continued with further follow-up. Among men, no similar weight effect of switching from TAF/FTC/DTG to TDF/3TC/DTG was observed. This study demonstrated that switching to TDF from TAF may decrease weight among women. However, this benefit would need to be weighed against the bone and renal toxicities of TDF.

Verburgh and colleagues used the ATHENA (AIDS Therapy Evaluation in the Netherlands) cohort to also address this question of whether weight gain with InSTIs or TAF, or both, is reversible with switching off these medications (Abstract 673). In this study, they focused on participants who had 7% or higher weight gain after switching to TAF and/or InSTI, an extent considered clinically significant. Weight and BMI of participants who discontinued TAF, InSTI, or both were compared with those of participants who continued TAF, InSTI, or both, using at least 1 weight measurement taken 3 or more months after discontinuation.

Overall, the researchers found that the change in weight at 24 months was −1.48 kg (95% CI, −4.24 to 1.27) after discontinuation of TAF (n = 21), −2.73 kg (95% CI, -6.22 to 0.66) after discontinuation of InSTI (n = 37), and −7.95 kg (95% CI, −15.57 to −0.33) after discontinuation of both InSTI and TAF (n = 11). In participants who continued TAF, InSTI, or both, weight change at 24 months after the first weight measurement to indicate a weight gain of 7% or more following the switch was −0.77 kg (95% CI, −1.32 to −0.21).

Based on this small study, it appears that weight gain after discontinuation of TAF, InSTI, or both was partly reversible and that weight was stable after the initial 7% or higher weight gain in participants switching to TAF, InSTI, or both. The ART medications to which these participants switched was not reported.

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Reviewer 1 reported consulting or advisor fees from Antiva, Assembly Biosciences, Generate Biomedicines, and IGM Biosciences, and fees for participation in review activities, such as data monitoring boards, statistical analysis, or endpoint adjudication committees with Gilead Sciences, Inc. (Updated June 30, 2023) Reviewers 2 and 3 reported no relevant financial relationships with ineligible companies. (Updated April 23, 2023)

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Invited Review

CROI 2023: Neuropsychiatric Complications in People With HIV

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Abstract. *The 2023 Conference on Retroviruses and Opportunistic Infections (CROI) featured new and impactful findings about neuropsychiatric complications in people with HIV and other infections. Reports included new evidence of (a) the importance of myeloid cells in the pathogenesis of HIV disease in the central nervous system, including as an HIV reservoir; (b) eukaryotic and prokaryotic viruses in cerebrospinal fluid during suppressive antiretroviral therapy; (c) the influence of sex on pathogenesis, including in novel neuropsychiatric biotypes identified by machine learning and other methods; (d) premature aging in people with HIV, including the brain-age gap observed on magnetic resonance imaging; (e) cellular and soluble biomarkers of neuropsychiatric complications in people with HIV; and (f) the neurotoxicity of certain antiretroviral drugs. This review summarizes these and other new findings and highlights new research directions for the neuro-HIV field.*

Keywords: HIV, CROI 2023, cognition, brain, CSF, depression, neurologic complications, neuroimaging, comorbidities

Introduction

The effects of HIV-1 in the central nervous system (CNS) were an important theme of several presentations at the 2023 Conference on Retroviruses and Opportunistic Infections (CROI). This summary is organized into 8 categories that highlight the substantial breadth of the data that were presented: pathogenesis of HIV disease in the CNS, persistence of HIV in the CNS, cognitive trajectories of people with HIV, aging and aging-related complications, neuropsychiatric biotypes, sex differences in neuropsychiatric complications of HIV disease, antiretroviral therapy (ART) and the CNS, and coinfections and the CNS. The exciting data this year inform new research opportunities as well as new implementation strategies to improve the health and welfare of people with HIV and other infections that affect the CNS.

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Pathogenesis of HIV Disease in the CNS

Substantial research supports the importance of myeloid cells, such as brain macrophages and microglia, in the pathogenesis of HIV disease in people with HIV. This research includes several reports that link CD14+CD16+ monocytes, a subset of circulating myeloid cells, to neurocognitive impairment in people with HIV,[1-5] possibly because they are more highly activated,[6] have higher HIV DNA content,[7] and migrate more readily across the blood-brain barrier[8] than other monocyte subsets. Veksler and colleagues built on these findings using specimens collected from participants in the Manhattan HIV Brain Bank, a member of the National NeuroAIDS Tissue Consortium (Abstract 486). They confirmed prior ex vivo findings by using a blood-brain barrier model to demonstrate greater transmigration of CD14+CD16+ monocytes in people with HIV who had neurocognitive impairment (particularly in working memory and speed of information processing) than in unimpaired people with HIV. This increased transmigration was associated with greater expression of CC chemokine receptor 2 on CD14+CD16+ monocytes. The authors also identified associations between higher levels of this cellular subset of myeloid cells and a higher glutamate/glutamine-to-creatine ratio, which can indicate imbalance in excitatory neurotransmission, in the left caudate nucleus using 1H-magnetic resonance spectroscopy.

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Another study evaluated the consequences of ex vivo infection of primary human microglia cells isolated from human postmortem brain tissue (Abstract 477). Dual-tropic envelope protein Morpheus–enhanced green fluorescent protein, an HIV construct encoding reporters for which expression was either HIV long-terminal repeat (LTR) dependent (heat-stable antigen and Cherry) or independent (enhanced green fluorescent protein) was used. The investigators found that more than 70% of the infected microglial cells harbored LTR-silent proviruses and that nonproductive HIV infection was 5 times more common than productive infection. Proteins that were secreted after infection were quantified by proximity extension assay. Infection with the construct resulted in significant microgliosis compared with controls, predominantly with LTR-silent infection that persisted 30 days after infection. Several markers were significantly secreted by infected microglia compared with controls, including vascular endothelial growth factor A, latency-associated peptide (LAP) transforming growth factor (TGF)-β1, urokinase plasminogen activator, colony-stimulating factor-1, and cluster of differentiation (CD)40, which provides evidence for the biologic mechanisms underpinning microgliosis in people with HIV and provides preliminary evidence for biomarkers of HIV infection of microglia in vivo.

The investigators found that more than 70% of the infected microglial cells harbored LTR-silent proviruses and that nonproductive HIV infection was 5 times more common than productive infection

Cross talk between microglia, astrocytes, and neurons was the focus of another presentation (Abstract 482). HIV latently infected microglia from the HC69 cell line that were cocultured with pluripotent stem cell–derived astrocytes had a significant reduction in HIV expression. A similar decrease in HIV expression was demonstrated when pluripotent stem cell–derived microglia cells were also cocultured with astrocytes. This occurred in an adenosine triphosphate–dependent manner that was abrogated by blocking adenosine production, but was reactivated with the addition of tumor necrosis factor (TNF)-β. The addition of astrocytes and pluripotent stem cell–derived neurons resulted in an even greater decrease in HIV expression.

Although CD4+ T cells are the primary reservoir for latent HIV, myeloid cells have been implicated as a secondary reservoir. An evaluation of monocytes and monocyte-derived macrophages from the blood of people with HIV taking long-term suppressive ART was performed with modified versions of the intact proviral DNA assay and the quantitative viral outgrowth assay (Abstract 419). Gag DNA was quantifiable from monocyte-derived macrophages from all participants, although levels were substantially lower than from CD4+ T cells. Within a subset of participants, quantifiable Gag DNA was repeatedly identified from monocyte-derived macrophages over several months. On the intact proviral DNA assay, latent HIV was frequently quantifiable from monocytes, although again levels were lower than for CD4+ T cells. Similarly, several participants had quantifiable latent HIV from monocyte-derived macrophages using the modified quantitative viral outgrowth assay, including a couple of participants who had repeatedly quantifiable levels over several months. Participants who had quantifiable latent HIV from monocyte-derived macrophages also had higher levels of HIV Gag DNA than those with undetectable HIV. This study provides strong evidence that myeloid cells can be a source of latent HIV that could reactivate.

In a rhesus macaque model of HIV, the effect of interleukin (IL)-15 antagonism was studied given its relationship to natural killer and CD8+ T cells (Abstract 479). To deplete these cell populations, 2 doses of rhesusized monoclonal antibody against IL-15 (or phosphate-buffered saline as a control) were given at days –21 and –7 prior to challenge with simian immunodeficiency virus (SIV) SIVmac239X, followed by necropsy at 7 or 14 days after infection. IL-15 neutralization of natural killer and CD8+ T cells resulted in higher SIV RNA levels in the blood but not in the brain, with a modest impact on barcoded virus variants in other tissues. However, IL-15 neutralization did appear to alter the brain immune response: IL-6+ perivascular and parenchymal microglia counts were substantially lower than in the control animals at 7 days as well as at 14 days in parenchyma only. In contrast, TGF-β+ perivascular and parenchymal microglia counts were substantially higher than in control animals at 7 days, with the difference persisting at 14 days only in the perivascular space. Although the reduction in IL-6 and increase in TGF-β in the absence of an increase in SIV RNA in the brain is reassuring, the observed immune changes could more easily allow establishment of a viral reservoir in the brain over a longer period of observation.

Several studies assessed plasma biomarkers as indicators of pathogenesis. Blackwell and colleagues examined associations between plasma biomarkers of neuronal injury, systemic inflammation, and innate immune activation and their relationship with changes in cognitive performance (Abstract 463). This study was performed among people with HIV and demographically similar people without HIV who were followed in the POPPY (Pharmacokinetic and Clinical Observations in People Over Fifty) study conducted in the United Kingdom and Ireland. Ten plasma protein biomarkers were measured: (1) neuronal injury biomarkers (neurofilament light chain, S100β); (2) systemic inflammation biomarkers (IL-2, IL-6, TNF-α); and (3) innate immune activation biomarkers (soluble CD14 [sCD14], IL-10, monocyte chemotactic protein-1 [MCP-1], soluble CD163 [sCD163], macrophage inflammatory protein-1 alpha [MIP-1α]). Within this cohort of predominantly virologically well-controlled White men, only biomarkers of innate immune activation (sCD14, sCD163, MCP-1), and not measures of neuronal injury or systemic inflammation, significantly differed between people with and people without HIV. For both groups, cognitive performance improved over time. Among people with HIV, changes in cognitive performance were associated with only MIP-1α and sCD14, with higher concentrations of each being associated with a worsening of cognition (global T-score) over a 2-year interval. These results suggest that innate immune activation and not neuronal injury or systemic inflammation differs between people with HIV and risk-similar people without HIV, and accounts for the continued cognitive dysfunction seen in people with HIV. Cooley and colleagues assessed neuronal injury (as measured by neurofilament light chain) in older, primarily Black people with HIV who had good virologic control. In this group, neurofilament light chain was associated with cardiorespiratory and physical health but not virologic or cognitive measures (Abstract 468). These results suggest that neurofilament light chain may not be a specific biomarker of cognitive performance, but instead may reflect cerebrovascular disease or metabolic changes seen in people with HIV. In a separate presentation, Cooley and colleagues also assessed the relationship between Alzheimer's disease (AD) plasma biomarkers (Aβ42/Aβ40 ratio, a clinically available blood-based biomarker for brain amyloidosis) and cognition in 4 groups of individuals: (1) cognitively impaired people with HIV; (2) cognitively unimpaired people with HIV; (3) cognitively unimpaired people without HIV; and (4) people without HIV who had symptomatic AD. Aβ42/Aβ40 ratios were low in people without HIV who had AD but not in the other groups (Abstract 487). A lower plasma Aβ42/Aβ40 ratio was also associated with smaller hippocampal volume but, again, only in individuals without HIV who had AD. Thus, the plasma Aβ42/Aβ40 ratio appears to differentiate cognitive impairment due to AD from other cognitive disorders in people with HIV.

After 32 months of antiretroviral therapy, HIV-infected cells decreased significantly within the lymph nodes but remained stable in cerebrospinal fluid

Persistence of HIV in the CNS

Single-cell profiling technologies continue to advance. In a pilot study of a single individual with chronic HIV infection before and after ART from the RV304/SEARCH (South East Asia Research Collaboration with Hawaii) study, Corley and colleagues evaluated blood, cerebrospinal fluid (CSF), sigmoid colon cells, inguinal lymph nodes, and T-follicular helper cells (Abstract 480). Before ART, lymph nodes harbored the highest frequency of HIV RNA-positive cells (3.75%). Less than 1% of all other cell types were HIV infected, with T-follicular helper cells being the least frequently infected (0.55%). After 32 months of ART, HIV-infected cells decreased significantly within the lymph nodes (to 0.03%) but remained stable in CSF (0.09%). HIV-infected cells appeared to express different genes than HIV-uninfected cells, and the genes expressed were different in blood than in lymph nodes (eg, *CD4*, *CD74*, interferon-stimulated gene of 20 kDa protein [*ISG20*], and others from blood and eukaryotic translation initiation factor [EIF], stathmin 1 [STMN1], and others from lymph nodes). To determine whether cryopreserved cells from CSF could be accurately used for these assessments, the cellular yield of fresh CSF was compared with that of cryopreserved CSF. Although the number of cells appeared to be similar, only fresh CSF had detectable HIV-infected cells. Based on receptor data, T-cell clones were shared across the compartments before and after ART, even though overall cell diversity was different across compartments.

In an ART interruption study, the authors evaluated CSF collected from 11 people with HIV, the majority of whom had viremia at the time of interruption (Abstract 478).

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Participants who had pleocytosis (CSF leukocyte count >5 cells/μL) during follow-up had a higher CSF-to-plasma HIV RNA ratio (P = .002). In the setting of pleocytosis, the CSF viral population was dominated by clonally expanded lineages, which were determined by single genome amplification or Illumina MiSeq. In contrast, the viral populations in blood and CSF were similar in the absence of pleocytosis. Using the assay for viral entry based on low surface density of CD4, the authors found that compartmentalized, clonal rebound of HIV in CSF was mostly T-cell tropic, but that CSF clonal rebound with pretherapy virus was rare. Pleocytosis was associated with higher CSF CXCL10 and matrix metalloproteinase-9 (MMP-9) concentrations but not with neurocognitive performance. Although corresponding results from blood during treatment interruption were not reported, the study results support the presence of a T-cell HIV reservoir in the CNS.

The development of single-copy assays has allowed for the identification of low-level HIV RNA in the central nervous system

The development of single-copy assays has allowed for the identification of low-level HIV RNA in the CNS. Single-copy assay results from the CSF and blood were evaluated in relation to soluble biomarkers, cognition, and depressive symptoms among people with HIV receiving ART with HIV suppression by standard assay (Abstract 485). Among 69 participants, 39% had less than or equal to 1 copy/mL of HIV RNA in plasma using a single-copy assay, and in a subset of 50 participants, 48% had less than or equal to 1 copy/mL of HIV RNA in CSF. Compared with participants who had more than 1 copy/mL, those who had less than or equal to 1 copy/mL of HIV RNA in either CSF or plasma had lower Aβ42 (in CSF and plasma), higher 8-hydroxydeoxyguanosine (in CSF and plasma), higher IL-6 (in CSF only), and higher total Tau (in CSF only). In addition, having less than or equal to 1 copy/mL of HIV RNA in plasma was also associated with higher plasma protein carbonyls, and having less than or equal to 1 copy/mL of HIV RNA in CSF was associated with higher CSF soluble TNF-α receptor II (sTNFR-II), lower CSF chemokine ligand 2 (CCL2), and lower plasma D-dimer levels. Having less than or equal to 1 copy/mL of HIV RNA in CSF, but not in plasma, was also associated with more depressive symptoms (P = .005). The use of either tenofovir alafenamide (TAF) (P = .003) or abacavir (P = .014) was associated with having less than or equal to 1 copy/mL of HIV RNA in CSF. Combined, the findings suggest that the combined pharmacologic and immunologic pressure needed to achieve very low HIV RNA concentrations during ART may have detrimental CNS effects.

The gut-brain axis was explored in an analysis of romidepsin for HIV latency reversal (Abstract 481). Neurocognitive performance was characterized with a panel of 6 tests, with impaired performance defined by a composite z score of -0.5 or lower. Three of 15 participants who had lower z scores before administration of romidepsin had stool that was enriched for certain taxa (including *Methanosphaera stadtmanae* and *Ruminococcus obeum*) but depleted of others (*Clostridium* species, *Paraprevotella*, and others). The lower z score group was also functionally enriched in 1,2-propanediol degradation (a pathway of propionic acid synthesis) before administration of romidepsin. An index of the significant taxa was created that decreased longitudinally from before romidepsin to the end of the study (P = .039) in participants with a lower z score. When the analysis was stratified by 2 study groups based on viremic control and the romidepsin intervention, *Desulfovibrio desulfuricans* was consistently associated with worse cognition, and *Parabacteroides johnsonii* was associated with more neuropsychiatric symptoms. The P-values for these findings were less than .05 after false discovery rate correction. This study expands on existing data on the gut microbiome and the CNS in people with HIV.

Cognitive Trajectories of People With HIV

Several studies longitudinally assessed the cognitive trajectories of people with HIV. Paul and colleagues studied the cognitive profile of people with HIV before and after starting ART (on average 6 days after diagnosis of HIV) in the Sabes study ("¿Sabes?" in Spanish means "Do you know?") in Lima, Peru (Abstract 460). Hierarchical longitudinal clustering identified 5 cognitive trajectory subgroups: Group 1 (16% of participants) exhibited above-average performance; Groups 2 (19%) and 3 (35%) performed within the average range; Group 4 (18%) exhibited mild difficulty in memory at baseline, with unimpaired performance on all tests by week 12; and Group 5 (12%) was the lowest-performing group (except for fluency), with scores that became unimpaired only by week 24. Each subgroup achieved unimpaired cognitive performance independent of the timing of ART initiation. These results confirm the findings of previous studies that starting ART soon after seroconversion leads to improvement that is sustained with continued viral control. Damas and colleagues examined cognitive

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performance over 4 years in people with HIV who were enrolled in the NAMACO (Neurocognitive Assessment in the Metabolic and Aging Cohort) study in Switzerland (Abstract 461). The authors focused on the changes in cognitive performance over time as defined by the mean yearly changes in global mean *z* scores from baseline. In this virologically well-controlled group of well-educated, predominantly White men with HIV, neurocognitive performance remained stable or improved over the course of 4 years. Executive function and sensory and perceptual skills particularly improved over time. The observed changes were not due to practice effects, as the tests were administered 2 years apart and different variations of tests were used.

Those with very-low-level viremia or low-level viremia performed worse on tests of memory and attention/working memory than those with effective viral control

The importance of good viral control was further confirmed by Trunfio and colleagues, who studied people with HIV receiving ART in Italy (Abstract 462). These authors assessed the impact of cognitive impairment on adherence as assessed by viral suppression. Participants were classified according to viral control as follows: (1) persistent very-low-level viremia (VLLV): HIV RNA values between not detected and 50 copies/mL at various, consecutive time points; (2) persistent low-level viremia (LLV): HIV RNA values between 50 and 200 copies/mL at various, consecutive time points; (3) viral failure: HIV RNA values greater than 200 copies/mL at various, consecutive time points; or (4) optimal viral control: either all HIV RNA values were not detected or only 1 HIV RNA value was greater than 50 copies/mL. Participants were predominantly White men, and those with VLLV or LLV performed worse on tests of memory and attention/working memory than those with effective viral control. Participants with viral failure performed worse in several cognitive domains than those with viral control. Asymptomatic neurocognitive impairment was associated with higher odds of VLLV or LLV (odds ratio [OR], 2.4; $P = .004$), and the odds were even higher in people with symptomatic neurocognitive impairment (OR, 5.2; $P = .001$). Although this was a longitudinal analysis, the authors did not address the sequence of the effects: Did neurocognitive impairment precede loss of viral suppression, perhaps by impairing memory and reducing ART adherence, or did loss of viral suppression precede neurocognitive impairment, perhaps by increasing immune activation and neuronal injury (or both)? The authors indicated that they are performing these and other analyses to address this issue.

Aging and Aging-Related Complications: Vascular Disease and Frailty

Petersen and colleagues studied the effects of comorbidities and social determinants of health on brain aging as assessed by neuroimaging (Abstract 186). This study was performed within a predominantly Black male group of people with HIV and people without HIV who underwent neuroimaging. A brain-age gap (BAG), defined as the difference between brain-predicted age and chronological age, was modeled as a function of clinical, comorbid, and social factors for these 2 groups. BAG was significantly elevated in people with HIV compared with people without HIV. Among people with HIV, worse BAG was associated with higher Framingham cardiovascular risk score, detectable HIV RNA level, and hepatitis C virus (HCV) coinfection. In subsequent models, BAG was affected by early-life stress and area deprivation index, a socioeconomic measure that combines geospatial data on housing, employment, education, and income. Educational attainment was linked with better BAG for people without HIV but not for those with HIV, consistent with a loss of resilience in people with HIV. Overall, these results suggest that additional comorbid conditions and socioeconomic factors are associated with brain aging along with HIV clinical metrics such as HIV RNA level.

Vascular disease occurs more frequently in people with HIV than in people without HIV and is associated with greater risk of cognitive and mental health disorders. For these reasons, Holroyd and colleagues evaluated relationships between Framingham risk score–based 10-year cardiovascular risk, estimated vascular age, and neurocognitive performance approximately 6 years after ART initiation during acute HIV infection in 356 virally suppressed participants in the RV254 project in Thailand (Abstract 464). Nearly two-thirds of participants had a higher estimated vascular age than their chronologic age, and greater vascular age deviation, defined as the difference between estimated vascular age and chronological age, was associated with higher CD4+ T-cell counts (mean, 0.5 years per 100 CD4+ cells/μL) but

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not with neurocognitive performance as assessed with a brief 4-test battery. One limitation of this project was that the incidence of cardiovascular events was low, likely because participants were generally young (mean age, 32 years at 288 weeks).

Investigators from the NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design) analyzed the relationships between vascular disease and mental health disorders (Abstract 145). This analysis included a 20-year period from 1997 to 2017 and focused on 2 types of myocardial infarction: type 1 (plaque rupture) and type 2 (demand ischemia). Among 33,071 participants, 49% had a diagnosis of anxiety or depression at baseline. A total of 869 participants subsequently developed myocardial infarction, with 57% of cases being type 1. In multivariable analysis, the diagnosis of depression, but not anxiety, at baseline was associated with incident type 1 myocardial infarction (OR, 1.23). Other covariates included male sex at birth, older age, tobacco use, diabetes mellitus, chronic kidney disease, and protease inhibitor use, as well as 2 covariates with ORs greater than 2 (hypertension and high cholesterol level or statin use). In contrast, the diagnosis of anxiety (OR, 1.42), but not depression, was associated with the occurrence of type 2 myocardial infarction. Older age, tobacco use, cocaine use, hypertension, diabetes mellitus, and detectable HIV RNA level were also associated with type 2 myocardial infarction, with chronic kidney disease (estimated glomerular filtration rate, <60 mL/min/1.73 m^2) having the strongest association (OR, 3.05).

Cerebrovascular disease has been linked to the presence of endothelial cell–derived microvesicles,[9] which can also be present in higher concentrations in people with HIV than in people without HIV.[10] Fandl and colleagues performed ex vivo experiments of human cerebral microvascular endothelial cells and endothelial cell–derived microvesicles that were isolated from the blood of people with and without HIV (Abstract 467). Compared with microvesicles derived from people without HIV, microvesicles from those with HIV were associated with greater inflammation (ie, greater release of IL-6 and IL-8), active endothelial nitric oxide synthase, and endothelin-1 production as well as impaired fibrinolytic capacity. If these events occur in vivo, they could increase the risk of cerebrovascular disease and stroke; thus, this may be another target for intervention.

In addition to the effects mentioned earlier, activation of myeloid cells, including CD14+CD16+ monocytes, influences vascular pathology and increases the risk of cardiovascular disease,[11,12] including carotid intima media thickness.[13] Based on findings on intermediate and nonclassical monocytes and work of their group on platelets,[14-16] Singh and colleagues compared platelet-monocyte complexes with an indicator of cerebral small-vessel disease (white matter hyperintensities on structural brain magnetic resonance imaging) in 110 people with HIV (Abstract 465). They found that people with HIV who had evidence of cerebral small-vessel disease had the highest levels of nonclassical monocytes and the strongest correlation between the circulating percentage of these cells and worse neurocognitive performance, compared with people with HIV without cerebral small-vessel disease and people without HIV. They also found that platelet-monocyte complexes had higher levels of numerous indicators of monocyte and endothelial activation (CCR2, CD40, P-selectin glycoprotein ligand-1 [PSGL-1], TNF receptor 2 [TNFR 2], and tissue factor) than noncomplexed monocytes. These findings are potentially impactful, because measurement of these cells may identify a subgroup of people with HIV whose brain injury is driven more by HIV and cerebrovascular disease than by other conditions. These cells could be targeted by therapeutic interventions.[17]

In multivariable analysis, the diagnosis of depression, but not anxiety, at baseline was associated with incident type 1 myocardial infarction

Frailty continues to be a common comorbidity in older people with HIV and has been associated with cognitive impairment in them.[18] Two presentations on frailty were presented from the multicenter Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort in the US. In the first, the authors compared the full Fried frailty phenotype assessment, which includes objective (strength and slowness) and subjective assessments, with a modified version in which the objective assessments were removed and a subjective mobility assessment was added to ease administration (Abstract 698). Among 522 participants, performance using the modified version significantly correlated ($\rho = 0.81$; $P < .001$) with that using the full version. The area under the receiver operating characteristic curve with the modified version was high for frailty (0.93) and prefrailty (0.86), and higher score on the modified version was also associated with falls in participants aged 55 years

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and older. The modified Fried frailty phenotype could be helpful if an in-person assessment is not possible. In the second CNICS report, the group evaluated comorbidities and symptoms associated with falls (Abstract 699). From a cohort of 2386 people with HIV, 435 (18.2%) reported having a fall in the previous 12 months. After adjustment for demographic factors, frailty was most strongly associated with an increased risk of falls, along with diabetes and self-reported symptoms of memory loss, fatigue, depression, neuropathy, and dizziness. People with HIV could be screened for these common neuropsychiatric symptoms (in addition to common comorbidities) to improve clinical assessments of fall risk.

Focà and colleagues from Italy also focused on falls, evaluating 1331 people with HIV aged 65 years and older (Abstract 700). Overall, they recorded 437 falls over a median of 3.4 years of follow-up, for an incidence of 0.67 falls per person-year. After adjustment for age, HIV infection duration, CD4+ T-cell count, HIV RNA level, and body mass index, multimorbidity (defined as at least 3 comorbidities) was associated with a substantially higher risk of falls (hazard ratio, 2.23; 95% CI, 1.19-4.21). The group also evaluated a subset of 311 people with HIV and compared them with 109 people without HIV who were also aged 65 years and older. After adjustment for age, sex, and multimorbidity, people with HIV had a higher fall risk than people without HIV (hazard ratio, 1.62; 95% CI, 1.07-2.46).

A key component of frailty is sarcopenia, or loss of muscle mass. A study from Thailand evaluated risk factors for sarcopenia in 277 people with HIV taking suppressive ART compared with 130 controls matched for age and sex (Abstract 696). Sarcopenia was defined by objective criteria (grip strength, walking speed, and muscle mass). Additionally, osteoporosis (by dual-energy X-ray absorptiometry scan), frailty (by Fried frailty phenotype), and nutritional status were assessed in the cohort, which had a median age of 55 years. People with HIV had higher rates of sarcopenia (8.3% vs 3.1%; $P = .05$), frailty (9.0% vs 3.1%; $P = .001$), malnutrition risk (18.0% vs 7.0%; $P = .002$), and HCV (9.0% vs 2.3%; $P = .011$) than controls. In multivariable models, several factors were associated with sarcopenia: male sex, body mass index less than 18.5 kg/m^2, HCV coinfection, prefrail or frail status, and malnutrition risk (all $P < .05$). Several of these factors are modifiable.

Brañas and colleagues also addressed frailty, reporting on longitudinally assessed sedentary people with HIV and people without HIV older than 50 years in Spain who were exposed to a 12-week multicomponent exercise program or a control program (Abstract 701). Those who completed the exercise program had improvements in anxiety and depression scores along with increases in muscle mass, strength, and aerobic endurance regardless of HIV serostatus. Overall, a multicomponent exercise program could lead to numerous benefits, including in neuropsychiatric symptoms.

After adjustment for age, sex, and multimorbidity, people with HIV had a higher fall risk than people without HIV

Neuropsychiatric Biotypes: Cognition, Depression, and Sleep Disturbances

Substantial research has focused on neurocognitive impairment in people with HIV, but other neuropsychiatric conditions such as depression and insomnia also commonly occur in this population. For instance, people with HIV are at greater risk than those without HIV for depression, including treatment-resistant depression. Such conditions can coexist in the same individual and can influence each other. To better understand this complexity, efforts have been made to combine these diseases into phenotypes (or biotypes) that might be more consistently linked to biologic mechanisms and therefore be associated with better response to therapeutic interventions.

Several presentations at CROI this year focused on depression. Meeder and colleagues analyzed multidimensional data from 1615 participants in the Dutch cohort study 2000HIV (Abstract 472). Participants completed assessments of substance use, depression, anxiety, impulsivity, sexual risk behavior, and quality of life, as well as ART adherence. In this cross-sectional analysis, the cohort had a low prevalence of symptoms of depression (6.1%) and anxiety (9.3%) compared with historical reports, but a unique aspect of this analysis was the inclusion of Ising network modeling, which indicated that symptoms of depression and anxiety were most strongly associated with impulsivity. More depressive symptoms were also associated with worse quality of life, and substance use was associated with more sexual partners and more sexually transmitted infections (STIs). Although these findings may not be surprising, they do support the use of assessments that extend beyond cognition alone and reinforce the need to implement additional measures in the clinic to better manage depression and substance use.

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An important and mostly unanswered question is what drives the greater risk of depression in people with HIV. Petersen and colleagues attempted to answer this question by comparing 6 soluble biomarkers in plasma from 150 people with HIV and 138 people without HIV who participated in research at the University of California San Diego (Abstract 475). Using factor analysis, they found that the 6 biomarkers loaded onto 2 factors, the first of which included IL-6, C-reactive protein, and D-dimer. This factor was associated with more depressive symptoms, and this relationship was modified by sex: men had a statistically significantly stronger association than women, particularly for IL-6. Rakshasa-Loots and colleagues also analyzed the relationship between soluble biomarkers and depressive symptoms in the COBRA (Comorbidity in Relation to AIDS) cohort and included several soluble biomarkers from both CSF and plasma (Abstract 476). These analyses included 125 people with HIV and 79 people without HIV. Like Petersen and colleagues, they found that IL-6 (in CSF) was associated with more depressive symptoms, along with TNF-α and monocyte induced by gamma interferon (or CXCL9) in plasma and MIP-1α (or CCL3) in CSF. Additional analyses provided evidence that these 4 soluble biomarkers mediated the relationship between HIV status and depressive symptoms, further supporting a role for inflammation in the depressive symptoms seen in people with HIV.

Additional analyses provided evidence that these 4 soluble biomarkers mediated the relationship between HIV status and depressive symptoms, further supporting a role for inflammation in the depressive symptoms seen in people with HIV

Two presentations focused on the relationship between ART regimens and depressive symptoms. One was hypothesis driven, focusing on the use of dolutegravir in 280 participants from the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) cohort (Abstract 471). The use of this integrase strand transfer inhibitor (InSTI) was associated with more depressive symptoms, and this association was modified by age, race, and use of antidepressants. People with HIV who used dolutegravir without an antidepressant had a level of depressive symptoms similar to that of people who used an antidepressant. Some of these associations are consistent with published reports (eg, older age[19]), but this is the first report to focus specifically on depressive symptoms and on use of antidepressants. Parra-Rodriguez and colleagues adopted a more discovery-driven approach in their analyses of data from 1538 participants in the WIHS (Women's Interagency HIV Study) (Abstract 469). A categorical transformation of data collected with the Center for Epidemiologic Studies-Depression scale indicated that 29.8% of participants were in a "high depression" group, that is, they had a value of at least 16 on at least 50% of assessments over time. Within this group, novel Bayesian machine learning methods showed that the combination of TAF with either a cobicistat-boosted InSTI or a protease inhibitor was associated with more somatic symptoms, such as poor concentration, sleep, and motivation. As cobicistat is not used to boost InSTIs other than elvitegravir, these findings differ from those that have implicated dolutegravir in neuropsychiatric adverse events. The observed association with TAF may be consistent with the previously mentioned report from Anderson and colleagues that identified associations between the use of TAF, single-copy HIV RNA suppression in CSF, and depressive symptoms (Abstract 485).

In addition to depression, neurocognitive impairment in people with HIV is associated with sleep disturbances, the focus of another set of analyses of data from the WIHS cohort (Abstract 473). A total of 337 women with HIV underwent neurocognitive testing and completed the Pittsburgh Sleep Quality Index questionnaire. About one-third met criteria for neurocognitive impairment, and in this subgroup, worse sleep quality was associated with worse neurocognitive performance. Additional analyses of components of sleep quality and cognitive domains indicated that mid-sleep waking was associated with poorer processing speed and executive function, bad dreams were associated with poorer processing speed, pain was associated with poorer working memory, and shorter sleep duration was associated with poorer attention and executive function. Another presentation summarized analyses of multidimensional data (objectively measured cognitive domains, depressive symptom subscales, subjective cognitive symptoms, and instrumental activities of daily living [ADLs]) from 1580 people with HIV in the CHARTER cohort using a 2-stage, unsupervised, machine learning clustering approach of self-organizing maps for dimension reduction followed by k-means clustering by Mahalanobis distance (Abstract 474). The goal was to identify novel phenotypes that are distinct from those

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typically identified based on neurocognitive testing alone. Analyses identified 4 phenotypes: a healthy group with good performance on the 17 analyzed features (38.5% of the cohort), a second group with a combination of mild neurocognitive impairment, moderate-to-severe depression, and mild impairment in ADLs (17.1%), a third group with mild neurocognitive impairment and very poor measurements on all other dimensions (12.9%), and a fourth group with mild-to-moderate neurocognitive impairment but largely without depressive or cognitive symptoms or impaired ADLs (31.5%). No data were presented to support that these phenotypes were more strongly associated with biologic indicators than, for example, neurocognitive impairment alone or that they may be associated with better response to therapeutic interventions, but the findings do support the potential importance of broadening our understanding of the various ways in which HIV and syndemic conditions may affect brain function.

An area of active investigation is the degree to which HIV-syndemic conditions, such as substance use and STIs, account for the brain-related complications seen in people with HIV, compared with HIV itself. For example, a published study showed similar prevalence of neurocognitive impairment in men who have sex with men (MSM) whether they had HIV or not.[20] Robertson and colleagues extended these prior findings by measuring 4 soluble biomarkers in CSF and blood in 135 participants (50 MSM with HIV who were taking suppressive ART, 50 MSM without HIV who were taking preexposure prophylaxis [PrEP], and 35 people who did not have HIV-related behavioral risk factors and who did not take PrEP ["controls"]) (Abstract 184). They found that both groups of MSM had higher levels of 3 of the 4 biomarkers than the control group (β_2-microglobulin, neopterin, neurofilament light), but they did not differ from each other. This important finding highlights the need to better understand the biologic effects of HIV-related behavioral risk factors such as substance use and STIs. Contributing effects of drugs used for PrEP must also be considered.

Sex Differences in Neuropsychiatric Complications of HIV Disease

Several studies addressed the influence of sex on neuropsychiatric complications in people with HIV. Chow and colleagues studied whether sex modifies the effects of traditional and HIV-related risk factors on stroke in people with HIV (Abstract 183). This group evaluated data from 5 CNICS sites that follow people with HIV who receive medical care. Strokes were adjudicated by neurologists. Among 13,584 people with HIV, there were 147 incident strokes during follow-up. Within this group, age but not sex was a risk factor for stroke, and a substantial age-by-sex interaction was observed. At younger ages, the risk of stroke was higher for women than for men. However, at older ages, women and men had similar risks of stroke. The risk of stroke in women was greater when they had a detectable HIV RNA level or used methamphetamine. These results suggest that additional risk factors for stroke, including viremia and drug use, should be considered for women, especially those who are younger.

Giron and colleagues studied the effects of long-term HIV infection on host glycomic alterations, including the loss of galactose (agalactosylation; measured as high levels of G-terminal ratio and G0 glycan groups), among men and women from the MACS (Multicenter AIDS Cohort Study)/WIHS Combined Cohort Study (Abstract 260). This study compared people with HIV on ART to people without HIV. HIV was associated with sex-dependent glycomic alterations: men and women had an induction of the proinflammatory agalactosylated glycans, but men had a reduction of anti-inflammatory sialylated glycans and women had a greater reduction of fucosylated glycans. HIV also accelerated the pace of age-associated agalactosylation. An increase in agalactosylation also correlated with inflammatory biomarkers of biologic aging and subclinical atherosclerosis. Overall, these results indicate new adverse, glycomic effects in HIV that appear to be sex dependent.

An area of active investigation is the degree to which HIV-syndemic conditions, such as substance use and sexually transmitted infections, account for the brain-related complications seen in people with HIV, compared with HIV itself

In addition to the effects of HIV, long-term ART may also play a role in these findings. Wells and colleagues studied whether sex-based differences affect the natural and treated history of HIV infection and immune responses within the ALLRT (AIDS Clinical Trials Group Longitudinal Linked Randomized Trials) cohort (Abstract 261). For a panel of 27 cytokines, the team did not observe significant differences in concentrations between men and women, with the sole exception of IL-18. For men and women, myeloid activation biomarkers were the ones that principally

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declined after initiation of ART. Leskov and colleagues studied whether shifts in innate immunity transcriptome signatures occur during the menopause transition and affect HIV pathogenesis (Abstract 262). The presenters noted that the latent HIV reservoir expands in women with HIV during reproductive aging. This reservoir expansion is accompanied by a shift of CD4+ T cells toward a more cytotoxic pro-inflammatory state that occurs during the premenopausal to perimenopausal transition.

Based in part on published data linking higher anti-cytomegalovirus (CMV) immunoglobulin G (IgG) levels to neurocognitive impairment[21] and higher Epstein-Barr virus (EBV) DNA levels in CSF to higher CSF neopterin levels,[22] Riggs and colleagues measured CMV and EBV DNA levels in peripheral blood mononuclear cells as well as anti-CMV IgG and anti-EBV viral capsid antigen IgG levels in plasma collected from 486 people with HIV who participated in cohort studies at the University of California San Diego (Abstract 491). Lower CMV DNA level correlated with worse neurocognitive performance, but only among women with HIV. The direction of this correlation was opposite to what was expected, which might be explained by the observation that lower CMV DNA level correlated with higher anti-CMV IgG level only in women. These analyses were limited to people with HIV who were taking suppressive ART and who did not have an acute coinfection. Henderson and colleagues described the correlates of CSF viral escape in 114 people with HIV who had a clinical indication for lumbar puncture (Abstract 185). One in 6 participants met criteria for CSF viral escape (ie, HIV RNA level in CSF greater than HIV RNA level in plasma), which was associated with the presence of ART drug resistance mutations and the use of ART drugs other than InSTIs. As in a prior publication,[22] the presence of EBV DNA in CSF was associated with CSF pleocytosis (median, 26 cells/μL) along with fewer CD4+ T cells, but EBV was not considered clinically related to any of the clinical conditions being evaluated (eg, neurosyphilis).

In addition to these more virus-focused analyses, Eden and colleagues from the University of Gothenburg presented new findings on an under investigated aspect of the host immune response, complement (Abstract 483). They measured components of the complement cascade (complement factor B, C1q, C3a, C4b2a, C5, C5a, and C3b) in CSF collected from 45 people with HIV and 28 people without HIV and found differences between the groups for components of all complement activation pathways, with generally lower levels in people with HIV. Lower levels would be consistent with complement consumption, perhaps by complexing with viral antigens or immune complexes. In people with HIV who were not taking ART, levels of complement components also correlated with neopterin levels in CSF, which in turn correlated with neurofilament light, 2 biomarkers that have been well linked to neurocognitive impairment in people with HIV. While small and cross-sectional, this project suggests that the complement system may influence the myeloid activation and neuronal injury that can occur in people with HIV.

ART and the CNS: Neurotoxicity and Novel Formulations

The potential neurotoxicity of ART continues to warrant investigation. Using a zebrafish model, Zizioli and colleagues evaluated dolutegravir exposure with and without folate rescue in relation to locomotor activity (Abstract 470). The group found that without folate rescue, dolutegravir-exposed embryos had substantially reduced locomotor activity, an effect that was abrogated by folate rescue. Raltegravir administration with or without folate did not impact locomotion. The group also evaluated neurogenin 1, a transcription factor that plays an important role in the development of dopaminergic neurons. In animals exposed to dolutegravir, neurogenin 1 expression was decreased in brain areas enriched with dopaminergic neurons, and spinal cord neurons that were peripheral projections of central dopaminergic neurons were consistently missing. This effect appeared to be strongest in the absence of folate.

Lower cytomegalovirus DNA level correlated with worse neurocognitive performance, but only among women with HIV

Structural modification of ART may reduce toxicity potential. A long-acting nanoformulation of dolutegravir was tested in the C3H/HeJ mouse model of pregnancy (Abstract 784). Intramuscular administration of nanoformulated dolutegravir resulted in maternal plasma dolutegravir concentrations in the blood similar to those of standard dolutegravir administration but was associated with a significantly lower dolutegravir concentration in embryonic brain tissue. Standard dolutegravir also led to less T1 relaxivity (indicative of more oxidative stress) on magnetic resonance imaging than that seen with nanoformulated dolutegravir, which was similar to that in control animals. Standard dolutegravir was also associated with significantly more changes in brain proteins than nanoformulated dolutegravir. While current guidelines endorse

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dolutegravir use in pregnancy, the results of this study support further research on dolutegravir nanoformulation.

In a study evaluating the effect of long-acting ART on myeloid cells (Abstract 427), rilpivirine and cabotegravir were loaded into lipid-wrapped polymeric nanoparticles expressing GM3, the CD169 ligand. The nanoparticle-ART regimen was retained in CD169+ monocyte-derived macrophages after almost 1 month in vitro and was associated with antiviral potency at this time point that was not present with the standard formulation of the drugs. In BALB/c mice, GM3 poly-lactic acid nanoparticles persistently colocalized with CD169+ macrophages in secondary lymphoid tissues, which did not occur with GM3-deficient nanoparticles. Lastly, treatment with GM3+ nanoparticle ART was associated with sustained virologic suppression for 3 weeks in bone marrow–liver–thymus humanized mice; this suppression did not occur with free drugs and was not as robust with GM3-deficient nanoparticles. Although the study did not evaluate brain tissue concentrations, it did demonstrate that nanoparticle ART could be tailored to reach specific cell types.

In another study evaluating the effect of long-acting ART on myeloid cells, bictegravir prodrugs were synthesized and then encased in nanocrystals in different formulations (dimeric: NMXBIC; monomeric: NMBIC, NM2BIC, and NM3BIC) (Abstract 540). These modifications allowed for enhanced hydrophobicity and lipophilicity without decrease in stability at 90 days. When tested in vitro with monocyte-derived macrophages, the drugs appeared to have minimal toxicity and preserved antiviral potency compared with standard bictegravir. Uptake and retention of all 4 nanoformulated drugs was high, with no loss of p24 inhibition after HIV-1ADA challenge. After a single intramuscular injection, the drugs were evaluated in BALB/cJ mice, Sprague Dawley rats, and rhesus macaques. Therapeutic bictegravir concentrations persisted long enough with the NMXBIC and NM2BIC formulations that the investigators concluded that they could be dosed every 6 months, which would substantially improve on the currently approved once daily dosing of bictegravir.

Coinfections and the CNS

Cryptococcal meningitis continues to be a devastating opportunistic infection worldwide in people with HIV. A trio of studies involving individuals with HIV and cryptococcal meningitis in Uganda were presented. In Abstract 489, CSF immune biomarkers reflecting different T-helper cell responses were evaluated in relation to survival in 480 individuals. Women were significantly less likely to survive than men over 18 weeks of follow-up (47% vs 59%; $P = .02$). Several CSF immune markers were lower in women who died than in women who survived, including TNF-α, CXCL10, and IL-10. IL-10 was also lower in men who died than in those who survived, whereas the only other biomarker that differed between the 2 groups of men was IL-15, which was higher in those who died. These data suggest that immune responses may differ in women and men with cryptococcal meningitis and may influence survival. In a second presentation (Abstract 748), neuropsychologic testing was performed in 210 participants 12 weeks after their first episode of cryptococcal meningitis in the ASTRO-cm (Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis) trial. A total of 72% of participants were neurocognitively impaired on an 8-test battery at 12 weeks. Compared with participants who were unimpaired at 12 weeks, these participants had lower Glasgow Coma Scale values, lower serum sodium levels, and more seizures at baseline. Individuals with impairment at 12 weeks also were less likely to have had sterile CSF at baseline (5.3% vs 13.8%; $P = .04$) and had fewer CSF leukocytes at day 7 (median, <5 cells/μL vs 25 cells/μL; $P = .03$). Clearly, more effective treatments for cryptococcal meningitis are needed to optimize neurocognitive outcomes as well as survival. One limitation to this study was that flucytosine, an important adjunct to amphotericin, was not used.

The third and largest of the analyses from Uganda involved 874 people with HIV with cryptococcal meningitis combined from the ASTRO-cm study and the AMBITION-cm (AMBIsome Therapy Induction Optimisation) study (Abstract 749). Total CSF protein was evaluated in relation to clinical characteristics, CSF immune markers, and survival. Participants who had a CSF protein level above 100 mg/dL at baseline had better survival at 18 weeks (log-rank $P = .02$) as well as a higher baseline CD4+ T-cell count ($P < .001$), a lower CSF cryptococcal fungal burden ($P < .001$), and a higher percentage of sterile CSF cultures at day 14 ($P = .02$). In addition, participants with elevated CSF protein level were more likely to have a Glasgow Coma Scale value below 15 ($P < .01$) and self-reported seizures ($P = .03$). Combined, these associations may be due to a stronger immune response to *Cryptococcus*, which might cause more symptoms during the acute illness, but then more rapid resolution of symptoms and survival. This conclusion was supported by higher CSF protein level being associated with higher CSF concentrations of multiple cellular and soluble biomarkers, including CSF leukocytes ($P < .001$), IL-1β, IL-1Ra, IL-6, CXCL8/IL-8, IL-17, granzyme B, CXCL1/GROA, and programmed cell death ligand 1 (all $P < .05$).

The impact of COVID-19 on people with HIV continues

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to be substantial. Data were presented from a study in Thailand in which 112 MSM were followed longitudinally (Abstract 188) after acute HIV infection. After baseline evaluation, which included brain magnetic resonance imaging as well as testing for cognition and mood, 54 of the 112 participants later developed COVID-19 (median follow-up, 79 weeks). Although the 2 groups generally did not differ in terms of demographics, those who developed COVID-19 had significantly smaller pallidum volume at baseline (false discovery rate–adjusted P= .025). In machine learning models, several brain region volumes (particularly the right brain) were associated with the development of COVID-19, including smaller right pallidum. More depression symptoms, higher IL-6 level, and amyl nitrite (poppers) use were also associated with the development of COVID-19. These imaging differences may translate into differences in risk-taking behavior between the 2 groups. A separate article in *Topics in Antiviral Medicine* reviews other presentations on COVID-19, including its neuropsychiatric effects.[23]

Detection of viral sequences in CSF did not relate to neurocognitive performance, depressive symptoms, or soluble myeloid and neuronal biomarkers in CSF

Another common coinfection in people with HIV is HCV. In another analysis from the Bangkok acute HIV cohort, 79 people with HIV acquired HCV after starting ART; 50 were subsequently treated with direct-acting antiviral agents and achieved sustained virologic response (Abstract 490). In addition to improvements in liver enzyme levels and CD4+ T-cell counts, sustained virologic response was associated with improvement on a 4-test cognitive battery (P = .004) as well as 1 measure of stress. This study adds more evidence of HCV treatment benefits in people with HIV that extend beyond the liver.

Based on the potential contribution of the human virome to HIV comorbidities and other diseases, Trunfio and colleagues evaluated CSF from 81 people with HIV receiving suppressive ART for viral RNA and DNA levels (Abstract 488). Fifty-eight of these samples had retrievable results for prokaryotic and eukaryotic viruses, and 25.9% had a CSF HIV RNA level greater than 20 copies/mL. The most common eukaryotic viruses identified in CSF were EBV, HCV, human herpesvirus-6, human papillomavirus-96 and -201, and Torque Teno virus. Meanwhile, 13 classes of prokaryotic viruses were identified, with Siphoviridae being the most abundant. Detection of viral sequences in CSF did not relate to neurocognitive performance, depressive symptoms, or soluble myeloid and neuronal biomarkers in CSF. However, CSF virome within-sample diversity (alpha diversity) was greater in participants with polymerase chain reaction-detectable CSF HIV-1 RNA level, lower CSF glucose level, and a CD4+ count of less than 500 cells/μL. These results were significant in correlational analysis as well.

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