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Highlights of the 2016 Conference on Retroviruses and Opportunistic Infections CME

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- Pathogenesis of HIV disease
- Epidemiology of HIV and HIV prevention efforts
- Neurologic disorders in HIV disease and their treatment
- Complications of HIV disease and HIV-related coinfection
- Viral hepatitis
- Antiretroviral therapy

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Presenter: Charles B. Hicks, MD, Duke University Medical Center—August 30, 2016

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Presenter: Ellen G. Chadwick, MD, Northwestern University—September 6, 2016

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Author: Linda M. Gorgos, MD, MSc, University of New Mexico

***Pneumocystis jirovecii* Pneumonia in the HIV-Infected Patient** NEW
Authors: Anuradha Ganesan, MBBS, MPH; Marc O. Siegel, MD; Henry Masur, MD

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Invited Review

CROI 2016: Basic Science Review

Mario Stevenson, PhD

The 2016 Conference on Retroviruses and Opportunistic Infections continued to maintain balance in the representation of different areas of research related to HIV/AIDS. The basic science category encompasses research on viral reservoirs and HIV cure, on cellular factors regulating the interplay between virus and host, and on factors that influence viral pathogenicity. Basic research on factors that influence the interaction between the virus and the host cell continues to unearth surprises with the identification of a new host antiviral factor. Further, research into the mechanisms of viral persistence reveals that there is much to learn about how HIV-1 is able to persist in the face of antiviral suppression.

Keywords: CROI, 2016, cure, viral reservoir, persistence, posttreatment control, cofactors, reservoir elimination, virology, pathogenesis

Viral Reservoirs and Persistence

As the research field embarks on finding a cure for HIV-1 infection, there has been substantial effort made in understanding how the virus is able to persist in the face of suppressive antiretroviral therapy, as was discussed at the 2016. Conference on Retroviruses and Opportunistic Infections (CROI). Although the level of viral suppression is profound and can be sustained for years, there is rapid recrudescence of plasma viremia shortly after treatment is interrupted. A stark illustration of this was provided in a study by Henrich and colleagues.[1] In this study, 2 HIV-infected individuals underwent reduced-intensity conditioning allogeneic stem cell transplantation and were transfused with donor cells that had normal CC chemokine receptor 5 (CCR5) alleles. Following transplant, HIV-1 DNA and RNA were undetectable in circulating CD4+ cells and plasma during suppressive antiretroviral treatment. Further, viral DNA was undetectable in gut-associated lymphoid tissue in 1 individual. Both individuals underwent an analytic treatment interruption and became viremic after 84 days and 225 days, respectively. In both cases, rebound in viremia was substantially delayed when compared with typical rebound of 1 week to 8 weeks after the interruption of antiretroviral therapy. The source of rebounding virus in these individuals is a topic of great speculation. There appeared to be a complete replacement of host CD4+ T cells (microchimerism testing indicated less than 0.001% of peripheral blood cells were of host origin). This suggests that there may be nonlymphoid reservoirs that maintain viral persistence in the face of suppressive antiretroviral therapy. Further studies of this kind are needed to reveal the long-lived cellular reservoirs that sustain HIV-1 in the face of viral suppression. In contrast to the outcome of this study, sustained HIV-1 remission was achieved by mild myeloablative allogeneic hematopoietic stem cell transplantation using donor cells that were homozygous for a 32–base pair deletion in CCR5 (the Berlin patient, Timothy Brown).[2] The difference here is that viruses emerging from any long-lived host reservoirs would be unable to encounter permissive host cells. This has emboldened the field of HIV research to devise strategies with which to achieve sustained remission without resorting to allogeneic stem cell transplantation.

The debate continues as to whether viral reservoirs that persist in the face of antiretroviral therapy are quiescent or whether a component of a viral reservoir can harbor the virus in an active state. The general consensus is that a reservoir that persists in the face of therapy predominantly comprises latently infected memory CD4+ T cells. Because the virus is in a latent state, it is believed that there are no viral proteins expressed within the latently infected cell that can render the cell vulnerable to killing by cytotoxic T cells. This provides the rationale for cure strategies that promote reactivation from latency, so as to render infected cells susceptible to attack by cytotoxic T cells or viral cytopathic effects.

Are viral reservoirs that persist under antiretroviral therapy quiescent or part of a reservoir harboring virus in an active state?

Data from Cartwright and colleagues (Abstract 22) challenged the notion that the viral reservoir is quiescent during suppressive antiretroviral therapy. The investigators examined whether depletion of CD8+ T lymphocytes would impact persistent viral reservoirs in simian immunodeficiency virus (SIV)-infected rhesus macaques receiving suppressive antiretroviral therapy. Thirteen rhesus macaques were infected with SIV_{mac239} and were placed on a 4-drug antiretroviral regimen 8 weeks postinfection. On entering an aviremic state, CD8+ T cells were depleted following intravenous administration of anti-CD8 antibody. On depletion of CD8+ T cells, there was a rapid recrudescence of plasma viremia in all treated animals. Viremia resolved on repopulation of CD8+ T cells, and this was found to be independent of repopulation

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by CD8+ natural killer cells. The investigators also demonstrated that the level of viremia following depletion of CD8+ T cells correlated with the number of SIV-specific CD8+ T cells prior to depletion.

This study provides intriguing evidence that, in the setting of suppressive antiretroviral therapy, a portion of the viral reservoir maintains the virus in an active state and that antiretroviral therapy and suppression of CD8+ T-lymphocytes cooperate to effect suppression of virus. This study underscores the rationale for the use of therapeutic vaccines in reservoir elimination, as well as checkpoint inhibitors to boost CD8+ T-cell activity in HIV-1–infected individuals taking suppressive antiretroviral therapy.

CD8+ T cells suppress viral reservoir activity during suppressive antiretroviral therapy.

A study from Lifson, Picker, and colleagues suggested the existence of sanctuaries of persistent HIV-1 replication based on the anatomic distribution of CD8+ T cells.[3] Data from this study indicated that B-cell follicles support persistent SIV replication, because CD8+ T cells are excluded from B-cell follicles. Folkvord and colleagues (Abstract 23) examined whether the relative compartmentalization of SIV in B-cell follicles (where CD8+ T cells are excluded) versus extrafollicular regions (where CD8+ T cells are abundant) would diminish on depletion of CD8+ T cells. Three chronically SIV-infected rhesus macaques underwent lymph node biopsy prior to and following antibody-mediated depletion of CD8+ T cells. Frequencies of SIV RNA–positive cells were determined by in situ hybridization. SIV RNA–positive cells were more abundant in follicular versus extrafollicular cells prior to depletion of CD8+ T cells (11-fold and 44-fold higher in follicular and extrafollicular cells, respectively). Further, there was an increase in SIV RNA–positive cells in extrafollicular tissue to the extent that the difference in frequencies in follicular versus extrafollicular regions normalized. This underscores previous findings that virus-specific CD8+ T cells dictate virus compartmentalization and that extrafollicular tissues, which are accessible to CD8+ T cells, are most susceptible to CD8+ T-cell suppression.

B-cell follicles, from which CD8+ T cells are largely excluded, present sanctuaries for viral replication free of the suppressive effect of virus-specific cytotoxic T lymphocytes. Given the possibility that CD8+ T cells play a key role in viral suppression, there is considerable interest in augmenting CD8+ T-cell responses in order to better suppress viral reservoir activity. One approach being explored utilizes antibodies that target the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) axis to reverse CD8+ T-cell exhaus-tion. Abstract 25 examined the impact of anti–PD-L1 antibody (BS936559) on immunologic and virologic profiles of 6 HIV-1–infected individuals taking suppressive antiretroviral therapy. Participants received a single infusion of an anti–PD-L1 antibody. Gag-specific CD8+ T-cell responses and plasma HIV-1 RNA levels were assessed over 28 days postinfusion. Owing to strong responses in 2 of 6 individuals, there was an overall trend of increases in the average percentage of HIV-1–specific CD8+ T cells, and there were no changes in plasma viral RNA or cell-associated viral RNA over 28 days postinfusion. This represents the first study to employ a PD-1/PD-L1 axis inhibitor in HIV-infected individuals taking suppressive antiretroviral therapy. Additional studies are needed to determine whether reversal of CD8+ T-cell exhaustion can impact viral reservoirs that persist in lymphoid tissue during suppressive antiretroviral therapy.

B-cell follicles act as potential sanctuary sites for viral persistence.

Previous studies have indicated that T follicular helper (Tfh) cells are the major compartment of CD4+ T cells for HIV-1 infection and replication in viremic individuals. Abstract 82 examined whether this observation also held true for aviremic individuals. The investigators compared the amount of viral RNA produced by CXC chemokine receptor 5–positive/PD-1–negative and PD-1–positive memory CD4+ T cells isolated from blood and lymph nodes of individuals taking long-term surpressive antiretroviral therapy. Levels of viral RNA were 800- to 1200-fold higher in PD-1–positive CD4+ T cells relative to the PD-1–negative cell population in lymph nodes and 5702- to 73,000-fold higher relative to CD4+ T-cell populations in blood. It is difficult to interpret from the level of viral RNA and viral outgrowth measurements the frequency of infection with this approach. Nevertheless, these data underscore the central role played by PD-1–positive CD4+ T cells in the lymph node as a major infected cell compartment for HIV-1–infected individuals taking suppressive antiretroviral therapy.

Leth and colleagues (Abstract 26LB) examined the impact of a therapeutic HIV immunization and a latency-reversal agent on viral reservoirs in HIV-1–infected individuals taking suppressive antiretroviral therapy. In a single-arm trial, 20 HIV-1–infected adults received 6 immunizations with the Vacc-4x vaccine, a peptide-based HIV-1 therapeutic vaccine that targets conserved domains on Gag p24, followed by 3 weekly injections of romidepsin. These researchers previously demonstrated that administration of romidepsin increased both plasma and cell-associated HIV-1 RNA.[4] Following therapeutic HIV immunization and romidepsin infusion, viral reservoirs were gauged by changes in total cell-associated HIV DNA and viral outgrowth assay in 17 individuals who completed all Vacc-4X immunizations and romidepsin infusions. Cell-associated proviral HIV DNA and viral outgrowth (evaluated in 6 of 17 participants) were each reduced by 40%. As this was a single-arm study, the relative contribution of Vacc-4x vaccines versus romidepsin injections to changes in reservoir activity cannot be determined. Nevertheless, this study underscores the need for additional trials that combine therapeutic immunization with latency-reversing agents to reduce the size of the viral reservoir.

Whitney and colleagues (Abstract 95LB) presented an update on ongoing studies aimed at evaluating the impact of an investigational Toll-like receptor 7 (TLR7) agonist (GS-986) in SIV-infected monkeys receiving an antiretroviral regimen. In 2015, these researchers presented intriguing evidence that administration of an oral TLR7 agonist induced transient plasma viremia. There was also a lower viral set point after interruption of antiretroviral therapy in animals receiving repeated doses of the TLR7 agonist. At CROI 2016, the investigators examined whether GS-986 and an investigational clinical compund (GS-9620) could affect viral reservoirs in SIV-infected macaques taking surpressive antiretroviral therapy. GS-986 induces interferon-α at doses that also induce transient plasma virema. However, low or undetectable interferon-α was induced by GS-9620. The investigators determined whether the effects of TLR7 agonists on plasma viremia could be uncoupled from induction of interferon-α. Eleven SIV_{mac251}-infected macaques underwent 65 days of treatment with surpressive antiretroviral therapy and maintained virologic surpession below 50 copies/mL of SIV RNA. Animals received either GS-986 or GS-9620 once every 2 weeks while maintained on supressive antiretroviral therapy. Peripheral blood mononuclear cell (PBMC)-associated SIV DNA, DNA in lymph nodes and colon, and SIV outgrowth were assesed following stimulation with concanavalin A ex vivo. Transient but inconsistent SIV RNA blips were observed in all animals treated with the TLR7 agonist from doses 3 to 10 but not after doses 11 to 19. On completion of treatment with the TLR7 agonist and prior to termination of antiretroviral therapy, SIV DNA levels were reduced in all tissues, as was the level of SIV induction relative to the control group. The kinetics of rebound in plasma viremia in 7 of 9 treated monkeys were similar to those of the control group. Intriguingly, in 2 animals, plasma viremia was undetectable for 60 days after discontinuation of antiretroviral therapy, and they were also negative for virus reactivation ex vivo. These important results support the rationale for the use of TLR7 agonists in elimination of viral reservoirs in HIV-1–infected individuals taking supressive antiretroviral therapy.

Siliciano and colleagues previously demonstrated that the vast majority of proviruses in HIV-1–infected individuals are defective and, further, that many cells harboring intact proviruses do not readily generate infectious virus, as measured by viral outgrowth assays.[5] In Abstract 83, Bruner and colleagues examined whether the fraction of defective proviruses increased over the course of infection. The fraction of defective proviruses was determined in individuals treated during acute or chronic HIV-1 infection. Provirus sequencing was conducted using full-genome polymerase chain reaction (PCR) assay following limiting dilution, and the number of intact proviruses was compared to levels of cell-associated viral DNA and viral outgrowth measurements. Less than 5% of proviruses were found to be intact in both acute and chronic infections. This indicates that defective proviruses accumulate rapidly following acute infection and is consistent with the rapid cytopathic effect of HIV-1 that would manifest in cells harboring biologically competent proviruses. The investigators also demonstrated that the majority of proviruses are predicted to be unable to make viral protein and, as such, are unlikely to be eliminated by purging protocols. However, it remains to be determined whether strategies that eliminate functional proviruses but not proviruses that are unable to make protein would produce a functional cure (ie, one that does not eliminate all virus from the infected individual but that leaves a viral reservoir that does not rebound if the infected individual discontinues therapy).

The issue of whether the viral reservoirs that persist in the face of antiretroviral therapy are nondynamic and quiescent or whether a proportion of the viral reservoirs retains some viral activity is of great debate. For example, cell-associated viral RNA persists in many individuals taking suppressive antiretroviral therapy, and the level of cell-associated RNA has recently been shown to correlate with time to viral recrudescence following an analytic treatment interruption.[6]

Does cell-associated viral RNA act as a surrogate for viral reservoir activity during antiretroviral therapy?

Hong and colleagues (Abstract 85) investigated the fraction of cells that harbor cell-associated viral RNA and the level of cell-associated RNA in individual cells from untreated individuals, viremic individuals, and aviremic individuals taking suppressive antiretroviral therapy. Unspliced viral RNA was examined by extracting RNA from multiple PBMC aliquots followed by dilution to end point or by cDNA synthesis and sequencing of cDNA molecules via single-genome sequencing and, additionally, serial dilution of PBMCs followed by measurement of HIV DNA and unspliced cell-associated viral RNA.

The frequency of proviruses expressing unspliced, cell-associated RNA was the same in viremic and aviremic individuals. In contrast, cells from viremic individuals had higher levels of cell-associated RNA. Because identical cell-associated RNA sequences were identified in multiple PBMC aliquots from aviremic individuals, this suggests that unspliced viral RNA is commonly expressed by expanded CD4+ T-cell clones. The investigators hypothesized that the higher level of cell-associated RNA in viremic individuals reflects a state of active viral replication, compared with cells obtained from aviremic individuals. An alternative hypothesis is that the high level of immune inflammation in viremic individuals creates conditions for more active transcription of proviruses. The authors also hypothesized that CD4+ T cells expressing unspliced viral RNA may contribute to viral recrudescence on interruption of antiretroviral therapy. It is unclear whether the cells that actively express unspliced HIV RNA in aviremic individuals are similar to those in latent viral reservoirs that persist in the face of suppressive antiretroviral therapy. It will also be interesting to determine to what extent cells that express unspliced HIV RNA contribute to the level of inducible virus that is measured using viral outgrowth assays.

As clinical studies aimed at eliminating long-lived viral reservoirs are developed, there is considerable interest in targeting individuals who initiated antiretroviral therapy early after infection. Such individuals may have less extensive viral reservoirs. Consistent with this, there is evidence that some individuals who initiate therapy during primary HIV infection exhibit virologic remission when treatment is halted. Gossez and colleagues (Abstract 87) examined the extent of post-treatment control in a cohort of individuals from South Africa and Uganda who initiated antiretroviral therapy during primary HIV infection and then subsequently underwent a treatment interruption. One hundred thirty-seven individuals within the first 6 months of seroconversion were recruited and randomly assigned to no treatment or 4 weeks to 8 weeks of antiretroviral therapy. Time to viral rebound following treatment interruption was compared with data from a previous UK cohort recruited under a similar protocol. Approximately 23% of those in the African study maintained an HIV RNA level below 400 copies/mL for 188 weeks following treatment interruption. This was a statistically significantly greater interval of viral remission than was observed among participants in the UK trial. Levels of viral DNA at treatment interruption were a predictor of time to rebound especially among UK participants. Given the substantial percentage of individuals who underwent remission following treatment interruption, this important study underscores the rationale for functional virologic cures that focus on individuals treated during primary HIV-1 infection.

"Shock-and-kill" protocols employ small molecules that are designed to reactivate HIV-1 production so that the host cell can be eliminated via viral cytopathic effects or CD8+ T-cell–mediated clearance. A number of small molecules including transcriptional activators and chromatin remodelers have been explored for the ability to reactivate latent HIV-1 in vitro and in vivo. Abdel-Mohsen and colleagues (Abstract 81) presented evidence that galectin-9 promotes reactivation of latent HIV-1. Galectin-9 is a β-galactosidase–binding lectin that exhibits a variety of biologic effects including tissue inflammation, T-cell immune exhaustion, and induction of apoptosis. Recently, galectin-9 was shown to be rapidly produced during acute HIV-1 infection and to remain at high levels during suppressive antiretroviral therapy.[7] Galectin-9 was also shown to augment expression of the cyclin-dependent kinase inhibitor p21, and it was shown that p21 regulates HIV-1 transcription.[8] Given this background, the investigators examined whether the impact of galectin-9 on p21 might lead to a reactivation of viral latency. The level of cell-associated HIV-1 RNA correlated with endogenous levels of plasma galectin-9. When galectin-9 was added to CD4+ T cells from HIV-1–infected individuals, there was an average 7-fold increase in level of cell-associated HIV-1 RNA, which was statistically significantly higher than for vorinostat-treated cells. Galectin-9 also induced the expression of apolipoprotein B mRNA editing enzyme catalytic subunit 3G (APOBEC3G), which reduced infectivity of nascent virus. Galectin-9 represents an attractive agent for reservoir elimination in that it can reactivate latent HIV and induce the expression of the antiviral restriction APOBEC3G. With this dual activity, any viruses produced from the reactivated cell would be rendered noninfectious after packaging of APOBEC3G in virions.

"Shock and kill" employs small molecules designed to reactivate HIV-1 production so that the host cell can be eliminated.

Virions trapped on follicular dendritic cells may represent another obstacle to HIV eradication.

A reservoir of latently infected memory CD4+ T cells is considered the single biggest obstacle to the elimination of HIV-1. However, it is unlikely to be the only obstacle, and it is possible that other reservoirs maintain viral persistence in the face of suppressive antiretroviral therapy. Carroll (Abstract 128) proposed that virions associated with follicular dendritic cells (FDCs) maintain infectivity for extended intervals and that these extracellular virions may constitute a viral reservoir for many months. Smith and colleagues demonstrated that when regular nonhumanized mice were passively immunized with an HIV-1 envelope antibody and subsequently injected with HIV-1, infectious virus could be recovered from lymph nodes 9 months later.[9] This remarkable study has gone relatively unnoticed, perhaps because of the difficulty in creating this scenario in a more physiologic setting. In support of this early study, Carroll presented evidence that human FDCs isolated from the lymph nodes of virologically suppressed individuals taking antiretroviral therapy harbored HIV-1 in a nondegradative compartment from which it could be transmitted to uninfected CD4+ T cells in vitro. Further, HIV-1 virions could be purged from FDCs with a soluble complement receptor. These studies present an extremely disconcerting scenario for the enterprise of an HIV cure. Complement-opsonized viral particles are taken up by FDCs and retained in an infectious form for extended intervals. Current strategies such as shock and kill would not impact the FDC reservoir. It is also to be expected that during uncontrolled infection, the extent of virion production could lead to massive deposition of infectious particles in FDCs, making a tough obstacle to eliminate.

HIV Virology and Pathogenesis

The replication of HIV/SIV is opposed by several cellular proteins, known as antiviral restriction factors. The most studied of these antiviral restriction factors include the cytidine deaminase APOBEC3G, tetherin/BST-2, and SAM domain and HD domain 1 (SAMHD1). APOBEC3 proteins are packaged within virions, and on infection, APOBEC3 promotes catastrophic G-to-A hypermutations in nascent viral complementary DNA. As a result, hypermutated genomes are degraded or incapable of producing functional viral components. Tetherin/BST-2

inhibits the detachment of virions from the surface of the virus-producing cell, thereby preventing it from establishing infection in a new target cell. SAMHD1 is a deoxyribonucleotide triphosphate (dNTP) hydrolase that reduces cellular dNTP to levels that are suboptimal for reverse transcription. These antiviral restriction factors have a profound impact on viral replication, and as a consequence, HIV and SIV have evolved counter defenses to obviate these obstacles to replication. The so-called viral accessory proteins all, in some way, counteract these antiviral restriction factors: APOBEC3 proteins are neutralized by the accessory protein Vif, which promotes proteasomal degradation of APOBEC3 proteins; tetherin is directed away from sites of virus assembly by Vpu; and SAMHD1 is neutralized by Vpx, also through facilitated proteasomal degradation.

Wu and colleagues (Abstract 136) presented evidence for a new antiviral restriction factor that is antagonized by the Nef protein. Nef has been shown to augment viral infectivity in a variety of cell models, but the mechanism for the enhancement of infectivity has not been defined. Two recent studies demonstrated that the host cell proteins serine incorporator 3 and serine incorporator 5 (SERINC3 and SERINC5) are inhibitors of HIV-1 infectivity and that Nef counteracts these antiviral effects.[10,11] Nef has been shown to be crucial for viral replication and pathogenicity in the SIV/macaque model, and individuals infected with Nef-deleted viruses exhibit a nonprogressive phenotype. How Nef mediates these effects has, despite 2 decades of research, not been defined. A number of studies noted an effect of Nef on viral infectivity that was relatively mild and depended on the nature of the cell from which virions were being produced. However, a clone of the CD4+ T-cell line Jurkat (E6.1) produced virus particles that were up to 100-fold less infectious in the absence of Nef.[12] Analysis of some of the cellular factors required for the infectivity in Jurkat (E6.1) cells indicated that infectivity enhancement may occur through host cell endocytic trafficking.

A possible mechanism for the impact of Nef on virion infectivity has been identified.

Further, the Moloney murine leukemia virus glycoGag protein, although unrelated to Nef, can also enhance HIV-1 infectivity. Proteomic analysis of HIV-1 virions produced in the presence or absence of Nef indicated that SERINC3 and SERINC5 were more abundant in virions produced in the absence of Nef. SERINC3 and SERINC5 are highly conserved from yeast to mammals and are predicted to contain between 10 and 12 transmembrane domains. Data presented at CROI 2016 underscored the finding that SERINC3 AND SERINC5 proteins are necessary and sufficient for the effect of Nef and glycoGag on HIV-1 infectivity. For example, inhibition of SERINC3 and SERINC5 expression eliminated the infectivity enhancement by both Nef and glycoGag, and the infectivity of Nef-deficient virions increased more than 100 fold when produced from cells lacking SERINC3 and SERINC5 expression. The precise mechanism through which Nef interacts with SERINC3 and SERINC5 is being investigated, but one possibility is that Nef promotes a redistribution of SERINC3 and SERINC5 from the plasma membrane to an intracellular compartment. Similarly, it is still unclear how SERINC3 and SERINC5 proteins inhibit particle infectivity, but it is possible that these proteins may impair the fusogenicity of virions. A central question is whether the impact of Nef on SERINC3 and SERINC5 accounts, in totality, for the requirement of Nef in viral replication in vivo. This will require identification of mutations in Nef that abrogate interaction with SERINC3 and SERINC5 proteins and of whether the mutants recreate the phenotype of viral variants deleted in Nef.

Abstract 139 provided new insight into the process through which HIV-1 gains access to the nucleus. Primate lentiviruses, including HIV/SIV, differ fundamentally from animal oncoretroviruses in that they are able to infect nonmitotic cells. For this reason, lentivirus vectors are used for transduction of nondividing target cells. During infection of the cell, viral nucleic acids are deposited in the cytoplasm of the newly infected cell in the form of a high-molecular-weight subviral complex, commonly referred to as the preintegration complex. This complex contains viral nucleic acids in association with viral enzymes, including reverse transcriptase and integrase, conferring on this nucleoprotein complex a molecular size approximating that of a ribosome. Viral reverse transcription proceeds within this complex, which must translocate to the nucleus to permit integration of virus with host cell DNA.

One of the mysteries in lentivirus biology is how this nucleoprotein complex is able to circumvent the nuclear envelope that is present in a nondividing cell. Frauke Christ and colleagues (Abstract 139) used HIV-1 virions, in which a fluorescently labeled integrase had been incorporated *in trans,* to follow conformational changes in integrase that occurred during nuclear transport of viral preintegration complexes. Fluorescence resonance energy transfer microscopy was then used to gauge the number of integrase molecules within a single preintegration complex, as well as conformational changes in the complex that accompanied nuclear entry. The investigators presented evidence that the number of integrase molecules in each preintegration complex was substantially reduced during nuclear entry. These investigators previously demonstrated that the host factor lens epithelium-derived growth factor p75 (LEDGF/p75) interacts with integrase to serve as a cofactor for integration.[13] On gaining access to the nucleus, integrase interacted with LEDGF/p75, which produced a rearrangement in the preintegration complex to allow integration. Collectively, these data indicate that the preintegration complexes undergo size reduction that enables them to pass through the nuclear membrane and further suggest that the nuclear pore may act as a molecular filter that exerts a sieving effect such that complexes that have undergone a specific adjustment in size are competent for nuclear entry. These studies are important for the design of novel antiviral agents that block the translocation of the virus to the nucleus.

The macrophage-microglial cell is the predominant if not exclusive virus-infected cell in the central nervous system

(CNS). How HIV-1 accesses the CNS is a topic of debate. It has been suggested that perivascular cells are infected in the periphery and subsequently migrate to the CNS, and it has also been suggested that trafficking of immune cells to the CNS may facilitate HIV entry. Alvarez (Abstract 141) presented evidence that SIV-infected brain macrophages can leave the CNS. The investigators introduced fluorescent superparamagnetic iron oxide nanoparticles (SPIONs) into the cisterna magna where they were taken up by phagocytic cells. Seven days after introduction of SPIONs into the cisterna magna, SPION-containing cells were found outside of the CNS and, particularly, in the cervical lymph node. This was observed in SIV-infected monkeys an in uninfected control monkeys. This is the first study to demonstrate that brain macrophages can migrate from the CNS into the periphery, thus creating the opportunity to seed tissues outside of the brain with viruses that have adapted to CNS replication.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

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Invited Review

CROI 2016: Hot Spots in HIV Infection and Advances in HIV Prevention

Susan P. Buchbinder, MD; and Albert Y. Liu, MD, MPH

The 2016 Conference on Retroviruses and Opportunistic Infections (CROI) highlighted hot spots in HIV infection. Men who have sex with men (MSM), transgender populations, people who inject drugs, fisherfolk, migrants, adolescents, and older adults are heavily impacted in a number of regions. Stigma contributes to risk behaviors and HIV acquisition across populations. HIV testing is a crucial first step in the HIV care continuum, and several large community-based surveys are underway in Africa to increase HIV testing, linkage to care, and uptake of antiretroviral treatment. Advances in preexposure prophylaxis (PrEP) featured prominently at CROI 2016. Two large efficacy trials of a vaginal ring containing the investigational drug dapivirine demonstrated efficacy and safety in preventing HIV infections in women in Africa. Data on the safety of long-acting injectable PrEP and several investigational PrEP drugs and formulations were also presented. Knowledge and use of PrEP among MSM in the United States appears to be increasing, and high uptake was seen among black MSM when provided as part of a culturally tailored support program. The use of broadly neutralizing antibodies for HIV prevention is a novel and promising approach to be evaluated in efficacy trials.

Keywords: CROI, 2016, epidemiology, HIV, hot spots, injection drug use, phylogenetics, preexposure prophylaxis, PrEP, prevention, incidence, transmission, men who have sex with men, MSM, transgender populations, fisherfolk, sexually transmitted infections, HIV testing, broadly neutralizing antibodies, vaginal ring, dapivirine, tenofovir alafenamide, TAF, microbicide

Hot Spots of HIV Infection

At the 2016 Conference on Retroviruses and Opportunistic Infections (CROI), investigators reported on current HIV hot spots of populations heavily impacted by high transmission rates, and proposed strategies for intervention.

Men Who Have Sex With Men

Men who have sex with men (MSM) remain one of the populations most heavily impacted by HIV disease worldwide. Hess and colleagues at the Centers for Disease Control and Prevention (CDC) presented estimates of the lifetime risk of HIV acquisition among all populations in the United States from 2009 to 2013 (Abstract 52). They estimate that the lifetime risk of acquiring HIV infection for men overall in the United States is 1 in 64, highest among black (1 in 20) and Hispanic men (1 in 48) and lowest among Asian men (1 in 174). Risk for women was substantially lower (1 in 227 overall), with similar racial and ethnic disparities: black (1 in 48) and Hispanic women (1 in 227) were at substantially higher risk than Asian women (1 in 883). When evaluated by risk group, MSM had a 1 in 6 lifetime risk, with the highest risk among black (1 in 2) and Hispanic MSM (1 in 4) and the lowest risk among white (1 in 11) and Asian MSM (1 in 14). Among people who inject drugs (PWID), the risk of acquiring HIV infection was 1 in 23 for women and 1 in 36 for men. Similarly, heterosexual women were at higher risk than heterosexual men (1 in 241 vs 1 in 473, respectively). Racial and ethnic disparities were also observed among PWID and heterosexual persons.

When analyzed by geographic region, the highest lifetime risks were observed predominantly in the Southern United States. When evaluated by age, MSM were at highest 10-year risk in their 20s and male PWID were at highest risk in their 40s and 50s, perhaps a reflection of late diagnosis rather than late increases in risk. Although estimates from 2009 to 2013 are more hopeful than those from 2004 to 2005, this type of analysis highlights the unacceptable racial and ethnic disparities that continue to exist. These data are a call to action to intervene with highly active prevention, particularly in at-risk black and Latino populations for whom preexposure prophylaxis (PrEP) should be made available.

Finlayson and colleagues reported on data from the 2011 MSM cycle of the National HIV Behavioral Surveillance, a venue-based time location sampling survey conducted in 20 US cities (Abstract 930). This analysis compared black MSM aged 18 years to 24 years with those aged 25 years to 44 years. Investigators reported that HIV prevalence was 21% among younger and 32% among older men in the survey. However, of men who were HIV seronegative or whose HIV serostatus was unknown, younger men were more likely than older men to report engaging in receptive anal sex at their

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last sexual encounter, and to have a sexual partner who was at least 3 years older or black. Among HIV-seropositive, black MSM, younger men were also more likely to have older or black partners and were less likely to be currently taking antiretroviral therapy. These data suggest that the high risk of HIV acquisition among young black MSM may be attributable to sexual networks that may contain sexual partners among whom HIV prevalence is high, some of whom are not taking antiretroviral treatment.

Stigma also figures heavily into populations of MSM globally. Lahuerta and colleagues presented data on HIV prevalence and factors associated with prevalent HIV infection in a cross-sectional survey conducted among 550 MSM in Bamako, Mali (Abstract 921). Overall prevalence of HIV infection was 13.7%, and 90% of those who were HIV seropositive were unaware of their infection status. In a multivariable model of factors associated with prevalent HIV infection, rejection of sexual orientation by family members was associated with an adjusted odds ratio (aOR) of 31.3. If lack of knowledge of HIV serostatus was accurately reported, these results suggest that rejection of sexual orientation by family members contributes substantially to risk behaviors and HIV acquisition.

Stahlman and colleagues presented data on a cohort of 1370 MSM recruited from Abuja and Lagos, Nigeria (Abstract 924). Men seeking sex partners online were more likely to become HIV infected during the study (aOR, 2.2; 95% confidence interval [CI], 1.5-3.2) and also more likely to report 1 or more types of perceived or experienced stigma (eg, having avoided or been afraid to seek health care services, being physically hurt, not feeling protected by police, or being blackmailed, verbally harassed, or scared to walk around in public). Although causality cannot be proven, it is possible that stigma may drive risky partner-seeking and sexual behaviors among MSM.

Holtz presented data on the high rates of acute and early HIV infection among a cohort of 977 MSM with negative results on HIV screening tests in Bangkok, Thailand (Abstract 927). Acute or early HIV infection was detected in 5.3% of these men, who were tested every 4 months. HIV infection was higher among men aged 18 years to 21 years (aOR, 2.6; 95% CI, 1.1-6.1) who tested positive for hepatitis A antibody (aOR, 5.6; 95% CI, 1.5-20.8) or hepatitis B core antibody (aOR, 14.1; 95% CI, 5.6-35.4). Men who reported inconsistent condom use with steady male sexual partners (aOR, 3.8; 95% CI, 1.8-8.3) and who had rectal *Neisseria gonorrhea*, (aOR, 7.5; 95% CI, 1.4-39.1) were also at greater risk of acquiring HIV infection. This suggests that among high-risk populations, more sensitive blood-based HIV tests will be required to detect early or acute HIV infection, during which treatment may limit immune destruction and the size of the HIV reservoir.

Solomon presented data on the high prevalence of HIV among wives of married MSM in India (Abstract 928). In a convenience sample of 149 MSM and their wives from 3 Indian cities, HIV prevalence was 47% (95% CI, 39%-55%) among the men and 27.5% (95% CI, 20%-35%) among their wives. Despite being recruited by their husbands for this study, 31.5% of the wives reported not knowing that their husband was having sex with men, and 34% reported learning of their partner's sexual practices seeing him having sex with a man. Only 15% of the wives reported that their husband had disclosed his sexual contact with men. These results point to the need to identify MSM and their wives in cultures in which marriage to women is common for MSM, to provide treatment for HIV-infected men and PrEP for uninfected men and women.

Transgender Populations

Poteat presented an overview of HIV in transgender communities worldwide (Abstract 79). She clarified for the audience that the term transgender applies to persons whose gender identity differs from the sex assigned at birth, irrespective of any medical, surgical, or other interventions. A 2-step method for assessing gender identity allows for accurate categorization: first asking the current gender identity, and then asking the sex assigned at birth. An estimated 0.3% of the US population (700,000 individuals) is transgender, and 0.1% to 0.5% of the population in Europe is transgender. In India, where *hijra* or "third gender" persons have been recognized for many thousands of years, an estimated 1 million to 6 million persons are living as *hijra*. Several Asian countries officially recognize a third gender on government documents.

As HIV prevalence is quite low among transgender men, with most large estimates being less than 1%, Poteat turned her attention to focus on transgender women, those who identify as women but were assigned male sex at birth. According to Dr. Poteat, several meta-analyses suggest that the overall HIV prevalence among transgender women is 19%

Approximately 700,000 transgender persons live in the United States, with an HIV prevalence among transgender women (identify as women but assigned male sex at birth) of 22%.

globally, compared with estimates of 12% among female sex workers and 13% among MSM globally.[1] In the United States, where the estimated overall HIV prevalence is 22% among transgender women, HIV incidence among black transgender women is double that observed among white transgender women. In sub-Saharan Africa, up to 23% of participants in studies of MSM self-identify as women. HIV prevalence is substantially higher among transgender women; for instance, a study in Lesotho found that 60% of transgender women were HIV infected, compared with 27% of cisgender women and 28% of MSM.[2]

Poteat covered biologic factors that could increase susceptibility to HIV infection among transgender women. Many transgender women use feminizing hormones, including 17-beta estradiol, at doses up to 4-fold greater than doses taken by cisgender women. Estrogens commonly used in birth control products are not recommended for transgender women, as they increase the risk of thromboembolic events. The impact of these hormones on rectal mucosa is unknown.

Poteat stated that only 2% to 15% of transgender women undergo any gender confirmation surgery and that virtually nothing is known about HIV acquisition through the neovagina, which is generally constructed of keratinized epithelium through a penile inversion procedure.

Interactions between feminizing hormones and antiretroviral drugs are also incompletely understood. Some HIV nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs) and protease inhibitors decrease estrogen levels, leading some transgender women to increase estrogen dosing to unsafe levels. The pharmacokinetics of tenofovir disoproxil fumarate (TDF) are affected by exogenous estrogen, although the impact of endogenous estrogens on PrEP efficacy is unknown. Of the 14% of participants in the iPrEx (Chemophrophylaxis for HIV Prevention in Men) trial who were transgender women, those who were receiving hormone therapy had substantial declines in TDF drug levels over time, although it is unclear whether this was a result of poor adherence or altered pharmacokinetics of TDF.[3]

Stigma is a major driver of high rates of HIV infection and poor outcomes among transgender women. Unemployment, poverty, and housing instability arise from stigma and contribute to these health inequities. Among the nearly 5400 transgender persons receiving care through the Ryan White HIV/AIDS Program in the United States, rates of viral suppression were statistically significantly lower than those of nontransgender persons receiving care through the program (74% vs 81%, respectively), and rates of viral suppression were 53% for transgender women aged 20 years to 24 years and 53% for transgender women who were unstably housed.[4]

Among HIV-seropositive transgender women, concerns about antiretroviral therapy and its adverse effects rank fifth in top health concerns, after 1) gender-affirming, nondiscriminatory care; 2) hormone therapy and adverse effects; 3) mental health care; and 4) personal care. An analysis of data from 400 transgender women participating in 9 demonstration projects indicated that those who received hormonal treatment from their primary practitioner had a 3-fold increased likelihood each of viral suppression and of having had a medical visit within the past 6 months. Poteat called for a research agenda for transgender persons to include understanding of drug interactions between hormones and antiretroviral drugs, understanding of comorbidities, integration of gender care with HIV prevention and treatment, and inclusion of transgender community members in all aspects of research.

People Who Inject Drugs

Brooks presented an overview of the explosive HIV outbreak in Indiana discovered in late 2014 (Abstract 132). He noted that new diagnoses of HIV infection among PWID decreased by 63% in the 10 years from 2005 to 2014, so that in 2014, only 6% of new diagnoses in the United States occurred among PWID. However, in late 2014 and early 2015, an outbreak occurred among PWID in Scotts County, Indiana. This county ranked lowest of all Indiana counties in life expectancy, with one-fifth of the population living below the poverty line and 9% being unemployed. As of February 1, 2016, 188 new HIV diagnoses have been linked to this outbreak, almost all of which were among white individuals whose ages ranged from 28 years to 42 years. Most of those who became infected were injecting oxymorphone, the expense of which led those injecting it to use small amounts, 4 to 15 times per day. No syringe access program was available in Indiana at the time. More detailed information on the phylogenetic analysis is discussed in the section on transmission networks.

Ramachadran and colleagues presented data on hepatitis C virus (HCV) transmission in Scotts County during the same time period (Abstract 149). Of 312 persons found to have HCV infection, only 25% were coinfected with HIV. Unlike the HIV epidemic in which a single virus was introduced into the population, several HCV variants were introduced into the PWID population over time, traced back to at least 2010. Phylogenetic analysis using Global Hepatitis Outbreak and Surveillance Technology (GHOST) enabled investigators to identify 23 clusters, of which the largest (130 persons) accounted for nearly half of detected infections. However, minimal overlap was found between HIV contact tracing and HCV networks, suggesting that transmission patterns may differ for HIV and HCV.

Brooks discussed the successful strategies used by local and state health authorities and the CDC to curb the HIV outbreak in Indiana, including syringe access programs, HIV care for those infected, and integrated services delivered within the community such as issuing identification documents, employment assistance, and medical care. He then pointed out that the national increase in deaths caused by drug overdose is now heavily contributed to by opiate prescribing; opiates were the cause of 30% of overdose deaths in 1999 and now account for 60% of overdose deaths. The CDC recently issued guidelines on opioid prescribing for chronic pain.[5] Brooks noted that multiple cities in Asia, Eastern Europe, and the United States, including New York City, New York, experienced increases in HIV prevalence among PWID from near zero to more than 40% within 1 year to 2 years. In contrast to previous outbreaks, the opiate epidemic and its attendant epidemic of hepatitis C appears to be occurring primarily in nonurban settings and predominantly in the southeastern United States. He closed with 3 recommendations to help jurisdictions prepare for similar outbreaks among PWID: 1) improve access to clean syringes and substance use diagnosis and treatment; 2) enhance capacity to detect a change in injection drug use within communities; and 3) prepare an action plan to rapidly address a similar outbreak, should one arise.

Opioid use now accounts for 60% of overdose deaths in the United States, up from 30% in 1999.

Another presentation from Perez and colleagues from the CDC evaluated the rates of opiate prescribing for HIV-seropositive individuals from 2009 to 2013 in the United States as

part of the Medical Monitoring Project (MMP) (Abstract 915). Perez noted that 21% of HIV-seropositive individuals in care through the MMP had been prescribed opiates during that time, with statistically significantly higher rates of prescribing in more vulnerable populations, including those living below the federal poverty limit, those with mental illness, and those with a history of injection and noninjection drug use. Practitioners must protect their patients from opiate overdoses through appropriate use and provision of naloxone to reverse overdoses. Practitioners should also be aware of the potential public health implications of prescribing opiates to individuals in vulnerable populations.

Fisherfolk

Several presentations focused on men and women working in the fishing industry in Kenya and Uganda, a population that is very highly impacted by HIV disease. Kwena presented an overview of the HIV epidemic in these populations (Abstract 171). He pointed out that 800 million persons worldwide depend on the fishing industry for their livelihood, with 80 million of those living in sub-Saharan Africa. He cited several studies of fisherfolk near Lake Victoria in Kenya and Uganda, in whom HIV incidence ranges from 2.4 per 100 person-years to 4.9 per 100 person-years. Kwena enumerated several drivers of high HIV incidence and prevalence in these populations, including high rates of mobility (40%-70%), transactional sex (42%-65%), and heavy alcohol use (52%-62%); HIV incidence is statistically significantly higher among fisherfolk who have these risk factors than among those who do not. He pointed to the dearth of services available to these populations and proposed that broad-based prevention and treatment services be provided in locations and at times that are convenient for the target populations.

High rates of mobility, transactional sex, and heavy alcohol use contribute to high HIV incidence and prevalence among fisherfolk in Kenya and Uganda.

Odongo and colleagues reported on HIV prevalence among 940 fisherfolk near Lake Victoria in Osembo, Kenya, from August 2014 to March 2015 (Abstract 903). HIV prevalence was higher among women than men (27% vs 18%, respectively; relative risk [RR], 1.5; 95% CI, 1.2-2.0). In multivariable analysis, prevalent infection was higher among those who had been previously married (aOR, 6.7; 95% CI, 2.9-15.7) or were currently married (aOR, 2.3; 95% CI, 1.1-4.9). Compared with those aged 30 years to 39 years, prevalent infection was lower among fisherfolk aged 13 years to 19 years (aOR, 0.1; 95% CI, 0.0-0.4) and 20 years to 29 years (aOR, 0.5; 95% CI, 0.3-0.7). Among those aged 20 years to 29 years, women were 3 times more likely than men to be HIV seropositive (24% vs 8%, respectively; $P < .05$).

Kagaayi and colleagues reported on the first and last of 4 rounds of the Rakai Community Cohort Study of more than 4000 residents of a large fishing community on Lake Victoria in Kenya (Abstract 986). They evaluated the impact of scale up of male circumcision and antiretroviral treatment regardless of CD4+ cell count from 2011 (before scale-up) to 2015 (after scale-up). Over that time, the circumcision rate for HIV-seronegative men increased from 30% to 54%, and antiretroviral therapy for HIV-seropositive men and women increased from 19% to 68% in this population. Over that same period, HIV seroincidence declined from 4.0 per 100 person-years to 2.9 per 100 person-years (aOR, 0.71; 95% CI, 0.44-1.15). These data demonstrate that substantial gains can be made in prevention and treatment in this highly affected population and that more remains to be done.

Migrants

Del Amo presented an overview of the magnitude of the HIV epidemic in migrant populations, with a focus on Western Europe and the United States (Abstract 173). In Europe, of the 83% of individuals with new HIV diagnoses from 2007 to 2012 for whom sufficient data on country of origin were available, 39% were migrants. Sub-Saharan Africans accounted for 53% of these migrants, Western, Central, or Eastern

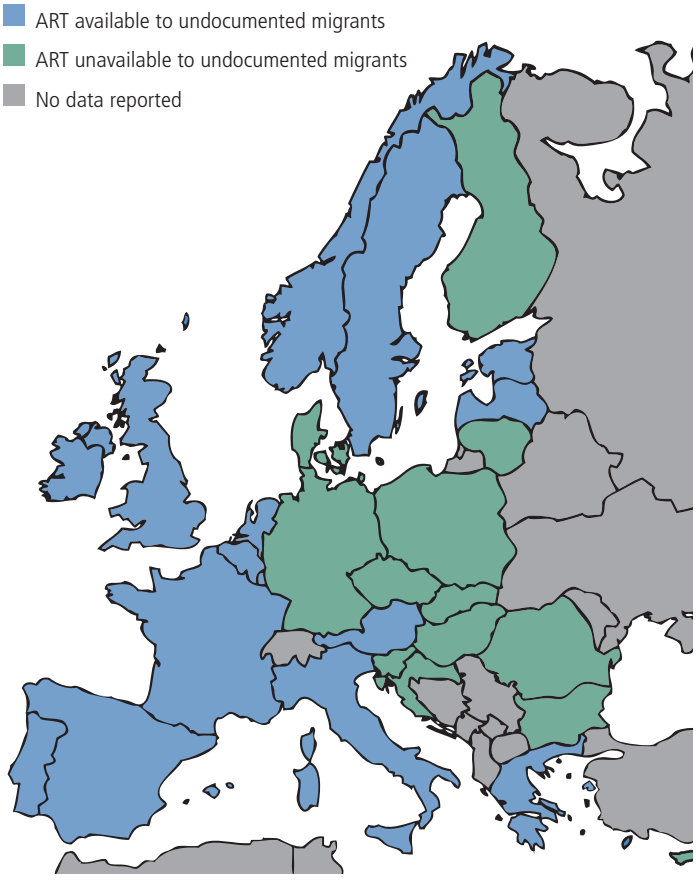

Figure. Provision of antiretroviral therapy (ART) for undocumented migrants in countries in the European Union and the European Economic Area, 2014. Adapted from European Centre for Disease Prevention and Control.[6]

Europeans for 21%, and Latin Americans for 12%. The number of HIV-seropositive migrants to Europe from Eastern and Southern Africa has been declining in recent years, although the number from West Africa remains stable. In the United States from 2007 to 2010, 16% of all new HIV diagnoses occurred in persons born outside of the United States: 41% from Central America, 21% from the Caribbean, and 14% from sub-Saharan Africa. Although most migrants were previously assumed to have been infected with HIV before arriving in Europe, current studies suggest that among newly diagnosed migrants, 75% of MSM and 50% of heterosexual men and women acquired HIV infection in the country of diagnosis. Limited access to testing and treatment likely contributes to later diagnosis (51% of migrants to Europe are diagnosed later) and poorer outcomes. Del Amo pointed out that only one-half of European countries provide antiretroviral drugs for undocumented migrants (Figure),[6] and asked the audience to consider the ethics of a policy of "test but not treat" for some populations.

Within-country migration can also drive local epidemics. Grabowski and colleagues presented data on nearly 23,000 men and women aged 15 years to 49 years who participated in the Rakai Community Cohort Study, which involved 38 communities in the Rakai district of Kenya (Abstract 902). From 2011 to 2015, 29% of participants migrated into or out of these communities, with the proportion varying from 9% to 49%, depending on the community. During this time, 25% of the total HIV-infected population and 57% of those newly diagnosed with HIV infection were migrants, predominantly women. Migrants were much less likely to be taking antiretroviral therapy than nonmigrants. These data provide further evidence of the vulnerability of migrant populations, even within the same country.

Adolescents

Sohn presented at a symposium on adolescents on the epidemiology of HIV infection in this population (Abstract 174). She pointed to a 2014 report by the World Health Organization that found that HIV infection was the leading cause of death among African adolescents and the second leading cause of death for youth worldwide.[7] Among young adults aged 15 years to 24 years, 4.3 million are living with HIV infection, although these estimates are often not divided by perinatally versus behaviorally infected persons. She made the case that perinatally infected youth have unique chronic health problems and long-term HIV management needs, account for a substantial proportion of mortality in this age group, and yet are rarely the focus of initiatives focused on HIV in youth. Only 16% of adolescents are known to be taking antiretroviral therapy according to a 2014 report from the Joint United Nations Programme on HIV/AIDS (UNAIDS),[8] likely based in part on the requirement of low CD4+ cell counts before initiation of antiretroviral therapy. A review of 20 studies of HIV-infected adolescents revealed viral suppression rates varying from 27% to 89%, reflecting substantial interpopulation differences in treatment and retention in care. For instance, a study in Swaziland found that 65% of those aged 10 years to 19 years were virally suppressed.[9] In contrast, the US Adolescent Medicine Trials Network for HIV/AIDS found low rates of viral suppression among HIV-infected adolescents aged 12 years to 26 years: 37% among those who were perinatally infected and 27% among those who were behaviorally infected.[10] Globally, HIV is the second leading cause of death among those aged 10 years to 19 years, the sixth leading cause of disability-adjusted life years, and is the only infectious disease in the top 10 disability causes. Sohn ended with a call to advocate for research and policy to improve outcomes for adolescents, and for more comprehensive and accurate data collection in this vulnerable population.

Worldwide, HIV infection is the second-leading cause of death among adolescents aged 10 years to 19 years.

Older Adults

Remera and colleagues presented data from the first national HIV household survey in Rwanda (Abstract 166). Previous estimates of HIV incidence have been based on models. The investigators suggested that these models substantially underestimate new infections. Models indicate approximately 5000 HIV infections per year, but the number is likely closer to 14,000 based on the national incidence of 0.27 per 100 person-years. In multivariate analysis, HIV incidence increased substantially with increasing age, and those aged 45 years or older were 4 times more likely to become infected than those aged 15 years to 24 years. Compared with single persons, widowers were 2 times more likely to become infected, but married persons were 70% less likely to become infected. The investigators also found unexpected and substantial regional differences, with persons from the Western Province having a 9-fold increased risk compared with those from the Northern Province. Results from this national survey can help shape policy, ensuring that older persons are one of the populations targeted by prevention strategies and that resources are targeted geographically.

Rosenberg and colleagues found older adults in a rural province in South Africa to be at substantial risk for HIV transmission and acquisition (Abstract 905). Among 5059 men and women aged 40 years or older who participated in the Health and Aging in Africa: Longitudinal Studies of INDEPTH Communities study, condom use decreased as age increased. In particular, more than 1 in 5 persons found to be HIV seropositive who reported they were HIV seronegative or that they did not know their serostatus also reported engaging in casual sex, and only 1 in 4 reported any condom use. The investigators concluded that HIV prevention interventions must also target older adults to reduce further transmission and acquisition in similar communities in sub-Saharan Africa.

Risk Factors for HIV-1 Transmission and Acquisition

HIV RNA in Semen

Mujugira and colleagues presented data on the presence of cell-free HIV RNA in semen samples of 231 African men newly initiating antiretroviral therapy (Abstract 164). Within the first 3 months after initiation of therapy, 24% of men continued to have detectable HIV RNA in semen, compared with 10% at 4 months to 6 months and 11% after 6 months. After full viral suppression in the blood was achieved, only 8% of men had detectable HIV RNA in their semen, with 82% of those having fewer than 1000 copies/mL. None of the men had sexually transmitted infections (STIs). In multivariable analysis, blood HIV RNA but not duration of antiretroviral therapy was a statistically significant independent predictor of seminal shedding.

Gowda and colleagues presented data on the risk of HIV transmission over time among 1833 HIV-infected persons enrolled in the University of Pennsylvania Center for AIDS Research Clinical Core Cohort Registry (Abstract 934). The investigators defined persons as being at risk of HIV transmission if their blood HIV RNA level was above 1500 copies/mL and if they reported engaging in sex without condoms. Overall, 9% of this cohort was at risk of HIV transmission over a median of 2 study visits. Risk factors for transmission included drug use, depression, younger age, and less than a high school education, identifying persons who may need additional support and prevention counseling.

Sexually Transmitted Infections

Sweat and colleagues reported on the risk of HIV acquisition after a diagnosis of primary or secondary syphilis in Shelby County, Tennessee (Abstract 929). Among 992 unique patients diagnosed with syphilis from 2005 to 2012, HIV incidence was 6.9 per 100 person-years. MSM had an incidence rate of 20.2 per 100 person-years and were 16 times more likely than heterosexual men and women to acquire HIV infection post-syphilis diagnosis in a multivariable model. This suggests that all individuals with a new syphilis diagnosis should be counseled about PrEP.

Several presentations focused on STI incidence among HIV-seropositive or -seronegative persons. Lachowsky and colleagues presented data on incidence rates for syphilis, *N gonorrhea*, and *Chlamydia trachomatis* infections among a cohort of MSM in Vancouver, Canada (Abstract 1007). Among 575 cohort participants, STI incidence was 17 per 100 person-years for HIV-seronegative MSM and 16 per 100 person-years for HIV-seropositive men. Compared with HIV-seronegative men, HIV-seropositive men were statistically significantly more likely to be diagnosed with syphilis (RR, 3.9; 95% CI, 2.0-7.8), but significantly less likely to be diagnosed with *N gonorrhea* or *C trachomatis* infection (RR, 0.6; 95% CI, 0.4-0.9). Goldberg Raifman and colleagues reported on case detection of *N gonorrhea* and *C trachomatis* in a multisite cohort of HIV-seropositive individuals in 7 US sites (Abstract 1005). The investigators found that in addition to increased screening in all risk groups from 2004 to 2015, case detection also increased in all groups. These studies support the need for regular STI screening for persons living with or at risk for HIV infection.

Two CDC presentations identified inadequate STI screening among HIV-seropositive persons in clinical care. Mattson and colleagues presented trend data on syphilis, *N gonorrhea*, and *C trachomatis* screening for HIV-infected men and women in the MMP, a national surveillance system that produces annual cross-sectional estimates of patients in care (Abstract 1004). The investigators found a significant increase in testing for all 3 STIs from 2009 to 2013 (20% to 36%; $P < .01$, for trend), but overall rates were substantially below the CDC recommendations of at least annual screening for all 3 STIs in HIV-infected persons. Patel and colleagues presented data on STI screening by anatomic site among MSM in the MMP from 2009 to 2012 (Abstract 1006). Of more than 6000 sexually active HIV-seropositive MSM, fewer than 1 in 3 were tested for *N gonorrhea* or *C trachomatis* at any site, and only 5% were tested for *N gonorrhea* or *C trachomatis* at extragenital locations. Positive results for *N gonorrhea* and *C trachomatis* were approximately twice as high in anorectal and pharyngeal sampling as in urine sampling.

Despite CDC recommendations for at least annual screening for STIs among people living with HIV infection, only 36% of HIV-infected individuals in care in the United States were screened for an STI in 2013, and only 5% of MSM were screened at extragenital sites.

Transmission Networks

Campbell and colleagues analyzed phylogenetic data from an outbreak of 181 cases of HIV infection among opioid users and their sex partners in Indiana (Abstract 215). From these analyses, the investigators were able to identify 3 separate clusters of transmission, with a solitary female bridge each between the central cluster and 1 other cluster. The researchers also identified how rapidly the outbreak spread during late 2014 and early 2015 and estimated that 50% of infections had already occurred before the first was diagnosed, 70% before a cluster was identified, and 85% before incident command was established to stop the epidemic. These data demonstrate the speed with which HIV can spread in networks of PWID, particularly among those who inject frequently and those who exchange sex for drugs.

Brenner and colleagues performed phylogenetic analysis of data from more than 4300 MSM newly diagnosed with HIV infection in Quebec, Canada, from 2002 to 2014 (Abstract 218). More recent infections belong to large clusters (10 or more linked transmissions) than in previous years: 29% of infections in 2002 to 2005, 34% in 2006 to 2009, and 46% in 2010 to 2014. Primary or early HIV infection was seen in

57% of large clusters but in only 26% of solitary transmission groups. This may be a result of rapid transmission of acute HIV infection, during which even a small increase in the average number of partners can substantially increase the interconnectedness of populations.

Ratmann and colleagues compared phylogenetic data from MSM diagnosed with HIV infection in the Netherlands from 2004 to 2007 with that from MSM diagnosed from 2008 to 2010 (Abstract 220). They found that men younger than 28 years contributed to a larger proportion of HIV transmissions and that most of these cases of transmission occurred when the young man was still undiagnosed. Although many of these transmissions occur between young men, young men were increasingly transmitting HIV to older men, pointing to the importance of early diagnosis and treatment of young MSM in this setting.

Green and colleagues demonstrated the importance of early partner services for newly HIV-infected persons (Abstract 224). Among 119 sexual partners of 574 persons with acute or early HIV infection, 33% were newly diagnosed with HIV infection and 24% had been previously diagnosed. Partners who were identified within 30 days of the index participant's HIV diagnosis were statistically significantly more likely to be newly diagnosed themselves, emphasizing the importance of early partner services for newly diagnosed persons, particularly those with acute or early HIV infection.

Wejnert and colleagues modeled HIV transmission from men to women in the United States (Abstract 1045). Based on data from 3 CDC surveillance databases, the investigators inferred that 33% of HIV transmissions to women come from MSM or from MSM who also inject drugs. Racial and ethnic disparities exist among these transmission estimates, with two-thirds of MSM-to-women transmissions occurring from black MSM and 20% from Latino MSM. These modeling data are estimates only but reinforce the urgent need to reach persons whose HIV infection has not been diagnosed or who have been inadequately treated for their infection, to address health disparities in both men and women.

HIV Testing

Because HIV testing is the first step in both prevention and treatment cascades, a number of abstracts at CROI 2016 focused on different strategies for increasing knowledge of HIV serostatus. Currently, several large community-based studies are underway to increase testing, linkage to care, and uptake of antiretroviral therapy.

Door-to-door, home-based HIV testing substantially increases awareness of HIV serostatus but also points to populations not adequately reached through these efforts. Shanaube and colleagues presented on the uptake of HIV testing in the HIV Prevention Trials Network (HPTN) 071 trial, also known as PopART (Population Effects of Antiretroviral Therapy to Reduce HIV Transmission), in Zambia (Abstract 981). Of the nearly 50,000 households visited by community HIV care practitioners, overall testing at the end of round 1 for those not known to be HIV-seropositive was 81% for women and 66% for men.

Casavant and colleagues presented data on home-based HIV testing in Mozambique (Abstract 975). Of more than 53,000 residents aged 15 years to 59 years, HIV prevalence overall was 20%, and 38% of these were new diagnoses. However, more women than men (52% vs 41%, respectively) were tested. Testing was also somewhat more common among older (age 25-59 years) than younger (age 15-24 years) residents and in rural than urban areas (50% and 45%, respectively, for each comparison).

A hybrid model of HIV testing, using community health campaigns (CHCs) followed by home-based testing for those not reached through CHCs, appears to achieve high coverage and to be cost-effective. Chamie and colleagues reported on the SEARCH (Sustainable East Africa Research in Community Health) study, a cluster randomized trial of HIV testing and treatment scale-up in 32 communities of 10,000 each in Kenya and Uganda (Abstract 979). From 2014 to 2015 (year 2), investigators repeated their hybrid HIV testing approach in the intervention communities and included additional services such as urgent care and men's health education in Kenya, and male circumcision, family planning, and cervical cancer screening in Uganda. Of the 77,778 community members who had lived in the community for at least 6 months in the previous year, 94% were tested at least once during the 2 rounds of testing, including 82% who received repeat testing. Half of the 11% of participants who were not tested in year 1 were tested in year 2, and 56% of those tested at home in year 1 participated in CHCs in year 2. This demonstrates the widespread appeal and broad coverage possible using this hybrid HIV testing model. An additional analysis reported by Zheng and colleagues found that of 4810 adults for whom there was detailed social network data, those with more dense local social networks were less likely to be tested for HIV infection through the hybrid approach (Abstract 985). This provides basis for strategies to augment the hybrid HIV testing model, to specifically target those who may be at greater risk but who do not take advantage of community-wide testing.

A hybrid model of HIV testing, using community health campaigns followed by home-based testing, achieved high coverage and was cost-effective.

Chang and colleagues estimated the cost of providing HIV testing through the basic hybrid model (Abstract 1062) and found that the cost per adult tested was $13.80 via CHC and $31.70 via home-based testing. Cost per HIV-seropositive adult identified varied based on HIV prevalence, with a range of $87 to $1245. Multidisease testing added little in marginal costs (eg, $1.16 per person for hypertension and diabetes screening, and $0.90 for malaria screening), suggesting that the benefit of multidisease services is quite cost-effective, particularly as it may reach some harder-to-reach

individuals than more traditional clinic or home-based testing models.

Partner notification for individuals newly diagnosed with HIV infection is one approach to identifying undiagnosed HIV-infected individuals, although care must be taken to prevent intimate partner violence that may result from disclosure of HIV serostatus. Cherutich reported on the effectiveness of partner notification services and the importance of early referrals in a cluster randomized study in Kenya (Abstract 50). Among 1305 partners of participants newly diagnosed with HIV infection enrolled in this study, immediate partner notification was more successful than delayed notification (3 months) by a substantial margin (71% vs 15%, respectively); immediate partner services also detected more HIV-seropositive partners (25% vs 5%, respectively) and was more successful at initiating care for HIV-seropositive individuals (16% vs 3%, respectively). Two new cases of intimate partner violence were reported and may have been study related.

Plotkin and colleagues reported on a feasibility study of partner notification services in Tanzania (Abstract 978). Three hundred ninety newly diagnosed, HIV-infected men and women were enrolled in the study from June 2015 to September 2015 at 3 hospitals in the region with the highest HIV prevalence in Tanzania; 3 potential index participants were excluded because of the risk of intimate partner violence. Most index participants chose to refer their sexual partners for HIV testing themselves (93%) rather than through their practitioner, and 57% of named partners presented for HIV testing. Of these partners, 62% were found to be HIV-seropositive and 63% of these were successfully enrolled in care. Most successful referrals (61%) occurred within 2 days, although an additional 19% were referred within 2 weeks. These studies suggest that immediate partner services may be most effective and will uncover both seroconcordant and serodiscordant partnerships. Active outreach to sexual partners may be most successful, although these studies did not conduct direct comparisons.

Several studies highlighted missed opportunities for earlier detection of HIV infection. Weissman and colleagues found that of more than 7000 persons diagnosed with HIV infection from 2006 to 2015 in South Carolina, 38% were late testers (ie, developed AIDS within 1 year of HIV diagnosis) (Abstract 965). The proportion of late testers declined from 41% of HIV diagnoses in 2006 to 2010 to 34% of diagnoses in 2011 to 2015 ($P < .0001$). Overall, 73% of late testers had had a health care visit in the 3 years before their HIV diagnosis, although 80% of these visits were in emergency departments. More than 2200 of those with a late diagnosis had been admitted to a hospital in the 3 years before their HIV diagnosis, suggesting that all hospitalized patients should be screened for HIV infection.

Hood and colleagues reported on uptake and perceptions of HIV testing and self-testing among 98 transgender women, 66 transgender men, and 956 MSM recruited during the Seattle Pride Parade in 2014 and 2015 (Abstract 971). Compared with MSM, transgender women and men were more likely to have an unknown HIV serostatus (4% vs 12% and 10%, respectively) and less likely to have undergone at least annual HIV testing in the past 2 years (58% vs 26% and 18%, respectively). Although 19% of MSM had ever used an HIV self-test, only 6% of transgender women and 3% of transgender men had ever used an HIV self-test. Additional strategies are needed to ensure high rates of HIV testing, particularly among transgender women.

Mugo and colleagues evaluated potential testing algorithms for persons with negative results on rapid HIV antibody tests but nonspecific symptoms consistent with acute HIV infection (Abstract 977). Investigators randomly assigned 410 participants to the standard of care (scheduling an appointment for the participant in 2-4 weeks) or an enhanced appointment intervention (utilizing Short Message Service [SMS], telephone, or in-person reminders to encourage participants to return for testing in 2-4 weeks). In multivariable analysis, investigators found that those randomly assigned to the enhanced intervention were twice as likely to return for testing as those given standard referrals for follow-up. Persons older than 25 years were 2.5 times more likely to return, regardless of treatment assignment, than younger adults.

Reaching Heterosexual Men

Ayles spoke about the importance of addressing men's needs when addressing the global HIV epidemic (Abstract 120). Data from the US Agency for International Development (USAID) Demographic and Health Surveys (DHS) program in 2013 documented that in many African countries, men are substantially less likely than women to have been tested for HIV infection. Although this is contributed to by perinatal testing and more frequent visits to clinics by women, many barriers exist for HIV testing of men, including the hours and environment in which testing is provided and the stigma that arises when HIV testing is the sole focus. The SEARCH study offers testing for numerous diseases (eg, diabetes, hypertension, malaria, tuberculosis) and separate spaces for men to be tested, resulting in testing of 90% of men. Home-based testing must be done during weekends and evenings to reach more men. Ayles showed data from the PopART study demonstrating that investigators were able to reach 56% more men aged 25 years to 34 years and 39% more men aged 35 years to 44 years when screening was conducted on Saturdays. Changing from a 9:00 AM to 5:00 PM schedule to an 11:00 AM to 7:00 PM schedule during weekdays increased screening among men aged 25 years to 34 years by 16%, and among men aged 35 years to 44 years by 29%. Ayles emphasized the need to care for men for their own health and not solely for their potential to transmit HIV to women.

Men in sub-Saharan Africa are less likely to know their HIV serostatus than women, but a hybrid model of broad community health campaigns followed by home-based testing resulted in a successful HIV testing rate of 90% in men.

In her oral presentation, Farquhar pointed out that testing of male sexual partners is important regardless of the HIV serostatus of pregnant women (Abstract 49). In addition to the benefit for the male partner, HIV-seronegative women are at substantially increased risk for HIV acquisition during pregnancy and breastfeeding, and HIV-seropositive women are more likely to accept prevention of mother-to-child transmission (PMTCT) measures if their male partners are tested for HIV infection and involved in care. Farquhar and her colleagues presented data from a randomized controlled trial of home-based partner education and HIV testing versus clinic invitation to male partners for uptake of testing, disclosure of HIV serostatus, couples testing, and identification of HIV-discordant couples. Of 601 women enrolled in the trial, 487 male partners had follow-up data available through 6 months postpartum, with approximately equal follow-up in the 2 arms. Overall, 87% of the male partners in the home-based testing arm were tested for HIV infection, compared with 39% in the arm that received clinic invitations. Among those who received home-based testing, women were more likely to be aware of their male partner's HIV serostatus (RR, 2.3; 95% CI, 1.9-2.7), to have been tested as a couple (RR, 3.2; 95% CI, 2.5-4.0), and to be identified as being part of a serodiscordant couple (RR, 3.4; 95% CI, 1.7-6.7). Although women at high risk for intimate partner violence were excluded from enrollment in the trial, 3% of women visiting at 6 weeks reported intimate partner violence; this was not considered by participants or staff to be related to study participation.

Preexposure Prophylaxis

Novel PrEP Formulations and Agents

Discussion of PrEP featured prominently at CROI 2016, which included a number of presentations on sustained delivery formulations of PrEP and novel PrEP agents. Two phase III studies reported efficacy results of a monthly vaginal ring containing the investigational NNRTI dapivirine for HIV prevention among women in Africa. Baeten and colleagues presented data from the ASPIRE (A Study to Prevent Infection With a Ring for Extended Use) study among 2629 women at risk for HIV acquisition in Malawi, South Africa, Uganda, and Zimbabwe (Abstract 109LB). Participants were randomly assigned to receive either a vaginal ring containing dapivirine 25 mg or a placebo ring, with a median of 1.6 years of follow-up. Enrolled women were young, less than half were married, and nearly half reported that they did not use a condom during their last sex act. Adherence was measured by concentrations of dapivirine in plasma and residually in returned rings. Overall, 82% of plasma samples had detectable dapivirine levels, at concentrations above 95 pg/mL, indicating at least 8 hours of continuous use, and 84% of returned rings had levels consistent with some use during the past month. There were 71 new HIV infections among those assigned the dapivirine vaginal ring (HIV incidence, 3.3/100 person-years), and 97 incident HIV infections among those assigned the placebo ring (HIV incidence, 4.5/100 person-years), resulting in a 27% (95% CI, 1%-46%; $P = .046$) RR reduction in HIV incidence. After excluding data from 2 sites with lower adherence in a predefined analysis, efficacy increased to 37% (95% CI, 12%-56%; $P = .007$).

Two phase III trials have demonstrated the efficacy and safety of the monthly dapivirine vaginal ring in preventing HIV infection among women in Africa.

In a post hoc analysis among women older than 21 years, efficacy increased to 56% (95% CI, 31%-71%; $P < .001$). HIV protection was not observed for women aged 18 years to 21 years, and adherence was lower among these women. The dapivirine vaginal ring was found to be safe, with adverse events well balanced between the study arms. Among women who seroconverted, detection of NNRTI resistance–associated mutations did not differ between the dapivirine and placebo arms (11.8% and 10.4%, respectively; $P = .80$).

In the same session, Nel and colleagues reported results from a sister trial, the RING study, conducted among 1959 women in South Africa and Uganda (Abstract 110LB). Participants were randomly assigned (2:1) to receive a dapivirine vaginal ring or a placebo ring over a 2-year period. Adherence was measured by concentrations of dapivirine in plasma and residual drug levels in used rings, similar to the ASPIRE trial. Overall, more than 83% of plasma samples and used rings indicated adherence. HIV incidence was higher than anticipated in the trial, and based on recommendations from an independent data and safety monitoring board, the final analyses were performed before the planned completion of the study. Enrolled women were young, and 89% were unmarried. There were 77 incident HIV infections among women who received the dapivirine ring (HIV incidence, 4.1/100 person-years) and 56 new HIV infections among women who received a placebo ring (HIV incidence, 6.1/100 person-years), resulting in a 31% (95% CI, 0.90%-51.5%; $P = .04$) reduction in the risk of HIV acquisition compared with placebo.

In a subgroup analysis of women older than 21 years, HIV protection increased to 37% (95% CI, 3%-59%), although efficacy was only 15% (-60% to 55%) among women aged 21 years or younger. Notably, HIV incidence was extremely high in this younger age group, with an HIV seroconversion rate of 8.2 per 100 person-years in the placebo arm. Furthermore, efficacy increased with lower residual levels of dapivirine in used rings (indicating increasing adherence), with 65% protection among women whose rings had residual levels of dapivirine of 20 mg or lower. These results strongly support higher levels of protection with increased ring use. Similar to the ASPIRE study, the dapivirine vaginal ring was found to be safe and well tolerated in the RING study, with similar rates of adverse events and drug resistance among those who seroconverted across study arms.

Chen and colleagues presented data on the safety and pharmacokinetics of the dapivirine vaginal ring for post-

menopausal women in the Microbicide Trials Network (MTN)-024/International Partnership for Microbicides (IPM) 031 study (Abstract 872). Women older than 50 years account for 12% of new HIV infections among US women, and postmenopausal women may be at higher risk for HIV infection owing to increased expression of CC chemokine receptor 5 (CCR5), decreased HIV-1 innate activity, low condom use, and low perceived risk. In this phase IIa study, participants were randomly assigned to monthly vaginal rings containing dapivirine 25 mg or placebo for 12 weeks. The mean age of the cohort was 57 years, with a mean age at menopause of 50 years. There were no differences in adverse events across the study arms, and only 2 women in the dapivirine arm chose to discontinue the vaginal ring because of adverse events. Levels of dapivirine in plasma were similar to those seen in women of reproductive age.

van der Straten and colleagues presented data on the acceptability and adherence of the dapivirine vaginal ring in the same cohort (Abstract 873). Overall, 99% reported that the ring was "very easy or easy to use," and 65% of women preferred the ring to condoms; 74% of participants reported that the ring was never out of the vagina, and 91% reported that the ring was never out of the vagina for more than 12 hours. Concerns about the ring (eg, discomfort during normal daily activities or the ring not staying correctly in place) decreased statistically significantly from baseline to month 3.

Adherence to the dapivirine vaginal ring was associated with higher levels of HIV protection.

In a symposium on innovations in PrEP, McGowan highlighted some of the potential promises and challenges of long-acting formulations of PrEP (Abstract 71). Long-acting formulations of medications have been used to improve adherence and address treatment fatigue across a range of fields, including contraception (eg, medroxyprogesterone) and psychiatry (eg, agents used to treat schizophrenia). Qualitative data on MSM indicate a hypothetical willingness to use long-acting PrEP agents and a preference for injections rather than a daily oral pill, and data on women in the VOICE (Vaginal and Oral Interventions to Control the Epidemic) trial indicate a preference for injectable, implantable, or ring-based prevention methods. Ideal attributes of a long-acting injectable drug include infrequent dosing interval (eg, 2-3 months), practical injection volume (eg, approximately 4 mL), and stable formulation without cold chain requirements. Potential challenges with long-acting injectable drugs include the long pharmacologic tail and the possibility of adverse events that would be difficult to manage given prolonged drug exposure. Additionally, HIV infection during periods of declining, subtherapeutic drug levels could result in the emergence of drug resistance, as was observed in 1 participant who received rilpivirine 300 mg, subsequently seroconverted, and in whom NNRTI resistance emerged. These issues have led to operational complexity in trial design for upcoming trials, which will involve a 1-month oral run-in phase to establish tolerability before initiation of injections and a 12-month period of oral PrEP after the last injection, to cover the pharmacologic tail.

Two drugs under investigation in long-acting injectable formulations are the NNRTI rilpivirine and the investigational integrase strand transfer inhibitor (InSTI) cabotegravir. Two phase I dose-escalation trials of rilpivirine were recently completed. Drug levels in rectal tissue were higher than those in vaginal tissue, and in explant challenge studies, HIV suppression was observed for rectal tissue but not for cervical or vaginal tissue. These findings were further explored in in vitro and explant studies conducted by Dezzutti and colleagues (Abstract 874). Rilpivirine penetrated colonic tissue more so than ectocervical tissue by more than 10-fold in vitro. Although concentrations achieved in rectal tissue were 5-fold higher than those needed to suppress viral infection in tissue explant studies, levels in cervical and vaginal tissue were 2.5-fold lower than suppressive levels. Based on these findings and additional issues of potential resistance and cold chain requirements, McGowan stated that it was unlikely that rilpivirine would advance into phase III trials for HIV prevention, although the drug is still being developed for HIV treatment.

Several researchers presented promising data on a long-acting, injectable formulation of cabotegravir at CROI 2016. Andrews and colleagues presented data on the effectiveness of PrEP with long-acting cabotegravir in an intravenous challenge model in rhesus macaques (Abstract 105). Two doses of long-acting cabotegravir 50 mg/kg given before and after intravenous challenge resulted in 7 of 8 animals being protected from simian immunodeficiency virus (SIV) infection through week 24, and 8 of 8 macaques were protected after a single dose given prior to injection. Further, 6 of 8 macaques given one dose of long-acting cabotegravir 25 mg/kg followed by one dose of long-acting cabotegravir 50 mg/kg were protected through week 24. Overall, long-acting cabotegravir provided 88% protection (21/24 macaques) against intravenous challenge, with lower concentrations of cabotegravir in plasma at the time of challenge among SIV-infected macaques than among those that remained uninfected. Investigators concluded that these results support the evaluation of long-acting cabotegravir as PrEP in PWID.

In the same session, Markowitz and colleagues presented data on the safety and pharmacokinetics of long-acting cabotegravir among 127 uninfected men at low risk for HIV acquisition in the ÉCLAIR study (Study to Evaluate the Safety, Tolerability, and Acceptability of Long-Acting Injections of the HIV Integrase Inhibitor GSK1265744 in HIV Uninfected Men) (Abstract 106). Participants were randomly assigned to receive cabotegravir or a placebo (5:1) in a 4-week oral phase, and following a safety assessment, eligible participants received 3 intramuscular injections of long-acting cabotegravir 800 mg or saline placebo at 12-week intervals. Eleven participants discontinued during the oral phase, 7 because of adverse events (mostly laboratory abnormalities). Long-acting cabotegravir was generally well tolerated; 7 participants discontinued during the injection phase, 4 because of injection site

reactions. Overall, 92% of participants who received long-acting cabotegravir experienced injection site pain, compared with 27% of those who received a placebo: approximately 50% were mild, 40% were moderate, and 10% were severe in grade, with injection site reactions lasting an average of approximately 5.4 days (compared with 2 days in the placebo arm). Pharmacokinetic data showed that peak levels of drug were higher and trough levels were lower than expected, such that 70% of participants had trough levels less than 4 times the protein-adjusted 90% inhibitory concentration, a level affording 100% protection in a macaque rectal challenge model. These findings likely reflect faster absorption from the depot injection site. Based on these results, an alternative dosing strategy of 600 mg every 8 weeks is being investigated in current studies of long-acting cabotegravir. Participant satisfaction with long-acting cabotegravir injections was high, with most participants favoring continuing injections of long-acting cabotegravir rather than taking oral cabotegravir.

Cabotegravir, a long-acting injectable PrEP agent, was well tolerated among HIV-uninfected men, and future efficacy trials are being planned.

Gulick and colleagues presented results from the HPTN 069/AIDS Clinical Trials Group (ACTG) 5305 study conducted among 406 MSM in the United States (Abstract 103). This phase II study evaluated the safety and tolerability of PrEP regimens containing maraviroc, a CCR5 antagonist that concentrates in the genital tract and is not commonly used for HIV treatment. HIV-uninfected MSM were randomly assigned to receive 1 of 4 treatments: maraviroc alone, maraviroc and emtricitabine, maraviroc and TDF, or TDF and emtricitabine, for 48 weeks. The rates of adverse events and study drug discontinuation did not differ among study arms. Study drugs were detectable in plasma in approximately 80% of participants at weeks 24 and 48. In a pharmacologic substudy among 72 participants, there were no significant drug interactions observed between maraviroc and TDF or emtricitabine. Overall, 5 participants seroconverted during the trial, all with R5 virus and no evidence of drug resistance; 1 seroconversion occurred in the maraviroc and TDF arm and the others occurred in the arm that received maraviroc alone. Two participants had undetectable drug levels at every study visit, and the other 3 had low or variable drug concentrations around the time of seroconversion. The investigators concluded that the maraviroc-based regimens were comparably safe and well tolerated to TDF and emtricitabine when used as HIV PrEP.

McGowan and colleagues presented data from a tissue substudy among 60 men enrolled in the HPTN 069/ACTG 5305 study (Abstract 104). Although in prior studies maraviroc was associated with increased gut-associated lymphoid tissue (GALT) T-cell activation and CCR5 expression in HIV-infected individuals, this study demonstrated no statistically significant changes in CD4+ T-cell activation or in CCR5 phenotype in any study arm. In colorectal explant HIV challenge studies, viral suppression was observed in samples from the 3 arms that received combination therapy through week 48, but viral suppression was not observed at week 24 or 48 in the arm that received maraviroc alone. McGowan noted that pharmacokinetic data on adherence are pending and also that previous studies have shown loss of maraviroc from explant tissue in culture, which could explain these findings.

Cranston and colleagues presented data from the MTN-017 study, the first phase II study evaluating the safety and acceptability of a rectal microbicide (Abstract 108LB). In this crossover design study, 195 MSM and transgender women were randomly assigned to different sequences of 3 study regimens, each taken for an 8-week period: reduced-glycerin 1% tenofovir gel daily, reduced-glycerin 1% tenofovir gel used before and after receptive anal intercourse, and daily oral TDF and emtricitabine. Rates of grade 2 or higher adverse events did not differ across study arms. Overall acceptability of the 3 regimens was high, with highest acceptability for the oral regimen: 90% acceptability in the arm assigned to oral TDF and emtricitabine, 80% acceptability in the arm assigned to gel used before and after receptive anal intercourse, and 70% acceptability in the arm assigned to daily gel use. There was a trend toward lower ease of use ($P = .08$) and lower intention to use product in the future ($P < .001$) for the daily gel compared with the oral regimen. Ease of use and future intention to use did not differ between the group that used gel before and after receptive anal intercourse and the group that used the oral regimen. Overall adherence to study product was high in all arms (>80%), as measured by drug detection in plasma, SMS diary, and product returns, but was lower in the daily gel arm than in the oral arm (OR, 0.35; 95% CI, 0.19-0.63; $P < .001$). The investigators concluded that rectal 1% tenofovir gel was safe when used daily or before and after receptive anal intercourse in this population and that use of gel in the latter instance was more acceptable. These results support further evaluation of reduced-glycerin 1% tenofovir gel for HIV prevention in men and women.

Given the low acceptability of applicators used to administer gel in rectal microbicide studies, Shieh and colleagues evaluated the rectal distribution of gels when applied as sexual lubricants compared with an applicator (Abstract 169bLB). Five HIV-seronegative MSM were administered 3.5 mL or 10 mL of ^{99}mTc-DTPA-radiolabeled hydroxyethyl cellulose (HEC) gel intrarectally using an applicator. After a washout period, the same participants were asked to self-administer radiolabeled wet gel lubricant to the anus manually, followed by simulated receptive anal intercourse with a phallic device. Single-photon emission computed tomography was performed 4 hours after each administration to measure gel distribution in the colon. The manual application method resulted in more variable distribution, compared with a more uniform distribution with an applicator. Further, only 3% of the initial dose was retained in the colon with manual application, compared with 88% and 95% of gel retained when an applicator was used for 3.5 mL and 10 mL, respectively. Additionally, manual application delivered 32-fold less volume of gel. These results suggest that manual application of a rectal microbicide gel may not

provide adequate drug concentrations or mucosal coverage for HIV protection, and application of gel as a lubricant may require distinct formulations to achieve higher antiretroviral loading.

Fast-dissolving vaginal films may provide more efficient vaginal drug delivery than gels, because they dissolve directly into vaginal fluid and are low cost. Bunge and colleagues presented data on the safety, pharmacokinetics, and pharmacodynamics of film and gel formulations of tenofovir (Abstract 871). In this study, 78 women were randomly assigned to receive 1 of 5 treatments: tenofovir 10 mg film, tenofovir 40 mg film, placebo film, 1% tenofovir gel, or HEC placebo gel. There were no differences in urogenital complaints across groups, and film users were less likely to report discomfort after insertion ($P = .02$) and product leakage ($P < .001$) than gel users. Median levels of tenofovir in plasma and genital tissue were comparable in the arms that received tenofovir 40 mg film and 1% tenofovir gel. In an ex vivo challenge model using cervical tissue, higher concentrations of tenofovir diphosphate correlated with lower HIV replication.

Fast-dissolving films may provide more efficient vaginal drug delivery than gels, because they dissolve directly into vaginal fluid and are low cost.

Tenofovir alafenamide (TAF) is an oral prodrug of tenofovir that achieves 90% lower plasma tenofovir exposure and is associated with fewer renal and bone toxic effects; however, its efficacy as a PrEP agent for HIV prevention is unknown. Garcia-Lerma presented data on the pharmacokinetics and efficacy of TAF and emtricitabine in preventing simian-human immunodeficiency virus (SHIV) infection in a repeat low-dose challenge model in macaques (Abstract 107). In a single-dose pharmacokinetic study, TAF 1.5 mg/kg achieved levels of tenofovir diphosphate in peripheral blood mononuclear cells (PBMCs) similar to those observed in human exposure; however, levels of tenofovir in plasma and of tenofovir diphosphate in rectal tissue were lower than those seen with TDF. In a repeated challenge model, 6 animals received TAF 1.5 mg/kg and emtricitabine 20 mg/kg orally 24 hours before and 2 hours after each virus challenge, and 6 received a saline placebo. All 6 macaques that received TAF and emtricitabine remained uninfected after 19 virus challenges, and all 6 that received a placebo became infected. Intracellular levels of tenofovir diphosphate and emtricitabine triphosphate achieved in PBMCs were high and accumulated approximately 2.5 fold and 1.5 fold, respectively, after 7 weeks to 14 weeks of dosing. These results support the development of PrEP with TAF and emtricitabine as an alternative to TDF and emtricitabine to prevent against rectal HIV infection.

Garrett and colleagues presented pharmacokinetic data on the genital and rectal tissue of women after a single dose of TAF (Abstract 102LB). Eight HIV-uninfected women were given a single dose of TAF 25 mg and had pharmacokinetic measurements taken over 14 days. In the first 48 hours, tenofovir exposure in plasma was 19-fold lower with TAF than with TDF, and tenofovir diphosphate exposure in PBMCs was 9-fold higher with TAF than with TDF, a finding consistent with prior studies. In mucosal tissues, tenofovir exposure was 2- to 10-fold lower with TAF than TDF, and tenofovir diphosphate exposure was 13-fold lower in rectal tissue and 1.3-fold lower in the female genital tract. Further, tenofovir diphosphate levels were below the limit of detection in 63% of rectal and 75% of genital tract samples from women who received TAF, compared with 0% and 25%, respectively, of samples from women who received TDF. These findings highlight the need for additional investigations on the pharmacology of TAF in mucosal tissues. Whether emtricitabine may be contributing to the protection observed with TAF and emtricitabine in the macaque challenge study (Abstract 107) merits further investigation.

Johnson reported results of early preclinical development studies of a long-acting, biodegradable, subcutaneous implant of TAF for HIV PrEP (Abstract 879). The research team developed a thin-film polymer device with a polycaprolactone membrane that controls drug release from a reservoir. In vitro prototype devices demonstrated linear release over 3 months, and release rates were tunable to estimated target ranges by changing device surface area and membrane thickness. TAF remained chemically stable in the device reservoir for at least 89 days, and ambient shipping and sterilization did not impact device performance. In vivo studies of this device are being planned.

Likely Breakthrough Infection on PrEP

Knox and colleagues presented a case report of HIV infection with multiclass HIV resistance in an individual taking PrEP (Abstract 169aLB). This individual, a 43-year-old man who has sex with men, seroconverted to HIV-1 after 24 months of successful PrEP, despite data suggesting long-term adherence to PrEP with TDF and emtricitabine. Pharmacy dispensing records showed consistent prescription refills, and levels of tenofovir diphosphate in dried blood spots tested 24 days after HIV infection was detected indicated consistent PrEP dosing in the preceding 1 month to 2 months. Further, tenofovir was detected in plasma on the day HIV infection was first detected, before the patient was aware of his HIV serostatus. Standard population and deep sequencing were performed on day 7 after HIV infection and demonstrated mutations conferring resistance to nucleoside analogue reverse transcriptase inhibitors (41L, 67G, 69D, 70R, 184V, 215E), NNRTIs (181C), and InSTIs (51Y, 92Q), suggesting transmitted rather than acquired drug resistance. Phenotypic drug resistance testing showed decreased susceptibility to all InSTIs

A likely breakthrough infection on PrEP occurred in an individual who reported high levels of adherence to PrEP and became infected with multiclass-resistant HIV while taking PrEP.

and mild reduced response to tenofovir (1.3×). Phylogenetic analyses revealed a very narrow range of sequence diversity, suggesting infection from a single source. Ritonavir-boosted darunavir and raltegravir were added to the individual's regimen on day 9 after detected HIV infection, and he achieved viral suppression by day 21. Subsequently, his antiretroviral regimen was optimized to dolutegravir, cobicistat-boosted darunavir, and rilpivirine, and his viral load has remained undetectable to date.

Bone and Renal Safety of PrEP

Oral PrEP containing TDF and emtricitabine has been associated with small decreases in bone mineral density (BMD). Grant and colleagues evaluated the recovery of BMD after PrEP was discontinued among MSM and transgender women enrolled in the iPrEx trial (Abstract 48LB). Among 498 participants enrolled in a dual-energy X-ray absorptiometry (DXA) substudy, decreases in BMD were observed during the first 24 weeks of PrEP use in those who had tenofovir diphosphate levels in PBMCs associated with a 90% reduction in HIV risk (16 femtomole/million cells). After PrEP was discontinued, average BMD recovered completely within 6 months in the spine and by the start of the iPrEx OLE (iPrEx Open Label Extension) study in the hip (a median of 1.5 years after PrEP was discontinued). There was a full recovery in BMD among younger (<25 years) and older individuals, and after adjustment for differences in study retention.

Several investigators reported on the renal safety of tenofovir-based PrEP in a themed discussion (Session TD-12). Gandhi and colleagues evaluated the relationship between cumulative levels of tenofovir and emtricitabine and declines in renal function in the iPrEx OLE study (Abstract 866). Among 1225 participants receiving PrEP, estimated glomerular filtration rate (eGFR) decreased modestly (2.5%) over 18 months. There was a monotonic relationship between percent decrease in eGFR and increasing quartile of tenofovir level in hair ($P = .008$), and the odds of eGFR falling below 70 mL/min (observed at 6% of person-visits) increased with increasing quartile of tenofovir concentration (OR, 4.4; 95% CI, 1.1-17.4) for fourth tenofovir hair quartile ($P = .045$, for trend); this event was more common in those with a baseline eGFR below 90 mL/min or those older than 40 years.

Liu and colleagues reported on changes in renal function in the US PrEP Demonstration Project (Abstract 867). Among 557 MSM and transgender women who received PrEP in STI clinics or a community health center, mean eGFR declined by 2.8% from baseline to week 12 ($P < .005$) and remained stable through week 48 ($P = .91$). Only 3 participants had TDF and emtricitabine stopped for elevated creatinine (>1.5× baseline per protocol); however, these elevations were not confirmed on repeat testing, and PrEP was restarted in all cases without further interruptions. Having levels of tenofovir diphosphate in dried blood spots consistent with taking 4 or more doses per week (≥700 femtomoles/punch) was associated with a statistically significant mean decline in eGFR of 4% at week 12 ($P = .01$). Similar to results seen in iPrEx OLE, a baseline eGFR below 90 mL/min and age older than 45 years were associated with eGFR falling below 70 mL/min. Together, these results support the safety of TDF and emtricitabine as PrEP and suggest that individuals with low baseline eGFRs and older individuals may warrant additional monitoring.

Mugwanya and colleagues assessed the frequency of proximal tubular dysfunction among HIV-uninfected African men and women in the Partners PrEP study (Abstract 868). In a subset of 1549 participants who received randomized treatment with TDF and emtricitabine or a placebo for at least 24 months and had urine and serum samples obtained, proximal tubulopathy (defined as having ≥2 of the following markers: tubular proteinuria, normoglycemic glycosuria, increased urinary phosphate, or increased uric acid excretion) was rare, occurring in 1.7% of participants who received TDF and emtricitabine and 1.3% of those who received a placebo ($P = .68$). Isolated tubular proteinuria and increased urinary excretion of uric acid occurred more frequently in those who received TDF and emtricitabine ($P < .01$, for each). In a case-control analysis, no association was observed between proximal tubulopathy and clinically relevant decline in eGFR (defined as a ≥25% decline in eGFR from baseline) ($P > .99$). The investigators concluded that monitoring urine markers of proximal tubular dysfunction is not an efficient approach to identify cases of tubulopathy, and suggested that creatinine clearance monitoring is sufficient for safety monitoring of kidney function in uninfected persons taking tenofovir-based PrEP.

PrEP and Sexually Transmitted Infections

In a symposium presentation, McCormack reported on trends in STIs in Europe, where PrEP has not yet been implemented (Abstract 69). Rates of *N gonorrhea* infection among MSM have increased sharply between 2008 and 2013, and rates of syphilis infection showed a similar trend during this period, all before the introduction of PrEP. Populations disproportionately affected by STIs in the United Kingdom include young women and men, black and mixed-race individuals, and MSM. McCormack highlighted 3 pillars of STI control: test, treat, and trace. Treatments for bacterial STIs have improved, although resistance to commonly used antibiotics is increasing. Important principles of treatment include having a short time from diagnosis to treatment (same day is ideal) and advising that sex be forestalled until treatment is completed or a test of cure is performed. For tracing infections, partner notification can be self-initiated or practitioner initiated. This strategy is usually preferred for regular sexual partners but can be difficult with anonymous sexual partners; however, internet and peer-facilitated notification may overcome some of these challenges.

Three pillars of STI control are testing, treatment, and tracing.

PrEP studies in Europe have been able to engage MSM at elevated risk for STI and HIV acquisition. In the PROUD PrEP

study among MSM who received PrEP in STI clinics in the United Kingdom,[11] 26% were diagnosed with rectal *N gonorrhea* infection and 21% were diagnosed with rectal *C trachomatis* infection in the year prior to enrollment (baseline STI rates were similarly high in the IPERGAY study[12]). In PROUD, the median number of anal sex partners at baseline was 10, and at 12 months of follow-up, participants that received immediate PrEP reported engaging in receptive anal sex without a condom with a greater number of partners than those that received deferred PrEP (P = .04). STI rates did not differ between the immediate and deferred arms during this initial period. However, there was a substantial increase in STIs in both arms in the second year of PrEP delivery, which may reflect temporal trends of increasing STIs. Despite high rates of STIs in PROUD and high HIV incidence in the deferred arm (9.0/100 person-years), PrEP was highly effective, resulting in an 86% reduction in HIV infections, with a low number needed to treat of 13. Since PROUD was completed, there have been more than 600 new HIV infections across the STI clinics, and more than half have been diagnosed in clinics in which PrEP could have been offered, highlighting important missed opportunities and the need for PrEP in this population.

Although the CDC recommends STI screening for MSM at least every 6 months, the optimal frequency of STI screening among MSM who are taking PrEP is unclear. Golub and colleagues presented data on STI diagnoses that would have been missed if participants had not received routine screening every 3 months in SPARK, a community-based PrEP demonstration project in New York City (Abstract 869). Among 280 participants who began PrEP, 21% were diagnosed with an STI in the 6 months before starting PrEP, and 43% were diagnosed with an STI after starting PrEP. Seventy-seven percent of STIs diagnosed at 3 months and 68% of STIs diagnosed at 9 months were diagnosed as a result of routine screening rather than symptomatic presentation. Rectal STIs, which are less likely to be symptomatic, were observed in 71% to100% of individuals with STIs at each visit. Among those with STIs, the proportion of individuals with repeat STI diagnoses increased steadily over time (33% at month 3, 77% at month 12).

Similarly, Cohen and colleagues reported data on potentially missed STI diagnoses among 557 MSM and transgender women enrolled in the US PrEP Demonstration Project (Abstract 870). A substantial proportion of gonorrhea (34%), chlamydia (40%), and syphilis (20%) infections would have been missed if screening had been conducted every 6 months instead of every 3 months as conducted in the study. Further, 83% of *N gonorrhea* and 76% of *C trachomatis* infections would have been missed without extragenital screening. Among visits in which at least 1 asymptomatic STI was diagnosed, 89% of individuals reported anal sex without a condom at the subsequent visit, with a median of 3 anal sex partners during this interval, suggesting substantial STI transmission potential among participants with asymptomatic STIs who are taking PrEP. Results from these 2 studies provide support for quarterly STI screening, including extragenital screening, among MSM taking PrEP.

Testing in Trials of PrEP

Several researchers reported data on the performance of different HIV testing algorithms in clinical trials of PrEP. Parikh presented data on the detection of acute HIV infection using fourth-generation combination antigen/antibody rapid tests in the VOICE trial, a large study of the safety and effectiveness of tenofovir-based products for HIV prevention among 5029 African women (Abstract 521). Among 229 presero-

Fourth-generation antigen/antibody HIV tests were more sensitive in detecting acute HIV infection in the setting of PrEP trials.

conversion samples tested for HIV-1 RNA, 68 had detectable HIV-1 RNA, of which 57 had a negative result by a third-generation rapid test. Among these 57 infections, a fourth-generation antigen/antibody enzyme-linked immunosorbent assay detected 33% more early infections than a third-generation HIV 1/2-O antibody-only enzyme immunoassay (EIA) (27 vs 8 infections detected, respectively). A Conformité Européene (CE)-marked fourth-generation rapid test detected 28% of infections missed by third-generation rapid testing, and the CE-marked test detected 21% more early infections than a US Food and Drug Administration (FDA)-approved HIV-1/2 combination antigen/antibody test (P = .0005). In contrast, HIV-1/2 antibody differentiation immunoassays and Western blot testing were insensitive (< 10%) in confirming acute HIV infections detected by fourth-generation testing. The investigators concluded that fourth-generation rapid testing with HIV RNA testing will be important for earlier detection of acute HIV infection in trials of PrEP.

Delaugerre and colleagues presented data on the usefulness of rapid tests for HIV diagnosis in the Agence Nationale de Recherche sur le Sida (ANRS) IPERGAY trial of PrEP (Abstract 522). Overall, 31 participants were diagnosed with HIV infection during the study. Nine were diagnosed at a preinclusion visit, 5 were diagnosed between preinclusion and initiation of PrEP, and 17 were diagnosed after initiating PrEP. Among 28 patients with stored sera samples, results of a fourth-generation combination antigen/antibody test were positive in 26 (93%) cases but missed 2 patients during acute HIV infection who had low HIV RNA levels (110 copies/mL and 450 copies/mL, respectively), compared with results of a rapid antibody test that were positive in only 15 (54%) cases. Western blot antibody titer and timing of diagnosis were used to determine stage of infection. The sensitivity of the rapid test was 100% for chronic infection, 78% for recent infection, and only 15% for acute infection (P < .002). These results support the use of fourth-generation assays to detect acute HIV infection early and to minimize the risk of selecting for drug resistance among individuals taking PrEP.

Bacon and colleagues reported on the performance of rapid EIA, fourth-generation antigen/antibody assay, and HIV-1 RNA testing in the US PrEP Demonstration Project (Abstract 524). Among 635 MSM and transgender women screened for the

study, 18 infections were detected at screening or initiation of PrEP, and 2 were detected during study follow-up. Although most HIV infections were detected using rapid EIA (14/15) and lab-based fourth-generation testing (13/13) at screening, acute infection was detected in 3 participants at initiation of PrEP using HIV-1 RNA testing only (120 copies/mL, 3343 copies/mL, and 51 copies/mL, respectively), and all 3 individuals had negative results on HIV rapid EIA and fourth-generation testing. Two participants became HIV infected during study follow-up; both had positive results on rapid EIA and fourth-generation testing. Both of these participants had low or undetectable drug levels at seroconversion and no evidence of drug resistance. Rates of false-positive results were low during follow-up for the rapid EIA (6/2680 tests) and fourth-generation antigen/antibody test (2/2673 tests). The investigators suggested that HIV-1 RNA testing should be performed before initiation of PrEP if available, particularly in individuals with recent HIV exposure, to detect acute infections early. In this cohort with high adherence to PrEP, rapid EIA and laboratory-based fourth-generation tests were adequate to detect HIV infection during follow-up.

Uptake, Coverage, and Delivery of PrEP

Black MSM are disproportionately impacted by HIV disease, comprising less than 0.4% of the US population but accounting for more than 20% of all new HIV infections in 2013. Wheeler and colleagues presented data on the uptake of PrEP and its use among black MSM in the HPTN 073 study (Abstract 883LB). This study enrolled 226 black MSM in Washington, DC, Los Angeles, California, and Chapel Hill, North Carolina, and all participants were offered 12 months of daily oral PrEP with TDF and emtricitabine along with client-centered care coordination, a theory-based approach to support adherence to PrEP by combining service-referral, linkage, and follow-up strategies to address unmet psychosocial needs. Overall, 40% of enrolled participants were younger than 25 years, 27% were unemployed, 31% were uninsured, and 31% had a history of incarceration. PrEP was accepted by 178 (79%) participants, and 68% remained on PrEP at 26 weeks. Self-reported adherence of greater than 50% was observed in 85% of participants at week 4 and 78% at week 26. Retention was high in the cohort, with 92% completing 12-month follow-up. Participants who initiated PrEP utilized a median of 6 client-centered care coordination sessions, compared with a median of 4 sessions among men who did not initiate PrEP. Five individuals who initiated PrEP seroconverted during 172 person-years of follow-up (HIV incidence 2.9/100 person-years; 95% CI, 0.9-6.8); 2 of these individuals discontinued PrEP before seroconversion. In comparison, 3 men who never initiated PrEP seroconverted during 39 person-years of follow-up (HIV incidence 7.7/100 persons-years; 95% CI, 1.6-22.5). These data suggest that theory-based, culturally tailored programs can potentially increase uptake and use of PrEP among black MSM.

Levy and colleagues evaluated correlates of uptake of HIV prevention interventions among black MSM in Washington, DC (Abstract 893). Two nonclinic-based samples were recruited, including a face-to-face sample of 75 black MSM with barriers to health care obtained through peer referral, and an internet-based sample of 93 black MSM regardless of health care status. The proportion of black MSM who received HIV prevention interventions was higher in community-based clinic settings (90%) than in primary (53%) or acute (44%) care settings ($P = .005$). In the internet sample, independent correlates of uptake of PrEP included being younger than 30 years (aOR, 5.51; 95% CI, 1.25-24.32), not having private insurance (aOR, 0.12; 95% CI, 0.02-0.69), trusting one's social network for advice about health issues (aOR, 5.65; 95% CI, 1.14-27.98), and having been offered an HIV test at last visit with a health care practitioner (aOR, 6.92; 95% CI, 1.25-38.16). Black MSM reported several structural barriers to accessing HIV prevention services in primary care settings, including stigma, difficulty disclosing sexual behavior without fear of judgment, and low cultural competence of practitioners. The investigators highlighted the importance of addressing these structural barriers to increase uptake of PrEP and other prevention interventions..

PrEP uptake was high among black MSM in the United States when provided as part of a culturally tailored support program.

Several poster presentations at CROI 2016 highlighted increasing knowledge and use of PrEP among MSM in the United States. Delaney and colleagues reported trends in awareness and use of PrEP from 3 nationwide internet surveys of US MSM between May 2012 and March 2015 (Abstract 889). Among a total sample of 10,097 MSM surveyed, awareness of PrEP increased from 45% in 2012 to 69% in late 2014 and early 2015; use of PrEP also increased substantially during this period and was higher among those at higher risk, including individuals with a recent bacterial STI (adjusted prevalence ratio (aPR), 2.45; 95% CI, 1.95-3.09) and those who reported having more than 10 sexual partners in the past 12 months (aPR, 3.47; 95% CI, 2.67-4.49). The prevalence of PrEP use was more than 10% in 4 US cities by early 2015 (New York City; San Francisco, California; Seattle, Washington; and Washington, DC).

Despite increasing knowledge about PrEP and interest in PrEP, there remains a large gap between those willing to use and those who have actually used PrEP. Scanlin and colleagues presented data on trends and correlates of recent use of PrEP among MSM in New York City participating in the Sexual Health Survey, a cross-sectional behavioral surveillance survey conducted from 2013 to 2015 (Abstract 888). Among 1572 respondents, use of PrEP in the past 6 months increased from 2.1% in 2013 to 14.8% in 2015. In bivariate models, use of PrEP was associated with being insured. In multivariable models, use of PrEP was associated with calendar year and several indicators of risk, including no con dom use at last sex, having an HIV-seropositive last sex partner, and engaging in sex without condoms with 3 or more

partners, or use of postexposure prophylaxis (PEP) in the past 6 months.

Mayer and colleagues reported on rates of PrEP use among MSM in Boston who received care at Fenway Health, the largest primary care center for MSM in Massachusetts (Abstract 890). Use of PrEP increased from 5 individuals in 2011 to 537 individuals in 2014 ($P < .001$), and 80% of individuals were still taking PrEP in 2015. During this period, 5 MSM who were taking PrEP became HIV infected (< 0.5%), compared with 93 (2.2%) MSM who did not initiate PrEP. STIs were common among PrEP users, and 36% of MSM who initiated PrEP in 2014 had a recent bacterial STI.

Novel approaches to increasing the capacity of practitioners to deliver PrEP are being evaluated. Edelstein and col leagues presented results of a public health detailing campaign to increase prescribing of PrEP among primary care and infectious disease practitioners in New York City (Abstract 892). Representatives from the New York City Health Department visited practices and provided short, individual-level presentations using the PrEP and PEP Action Kit at an initial and then a follow-up visit. Among 881 practitioners, 18% prescribed PrEP at initial visit (early adoption), and 13% prescribed PrEP after initial follow-up visit (incident PrEP prescribing). Early adoption and incident PrEP prescribing were more likely among infectious diseases practitioners, suggesting a high level of willingness to prescribe PrEP among these practitioners. Early adoption was also associated with working in a community health clinic and having a history of prescribing PEP, suggesting that prescribing PEP may be a gateway to prescribing PrEP; this finding supports the promotion of PrEP and PEP simultaneously in detailing efforts. Incident PrEP prescribing was associated with having an initial visit of 10 minutes or longer, suggesting a potential impact of public health detailing and supporting further evaluation of this innovative approach.

Patel and colleagues described missed opportunities to prescribe PrEP in primary care settings and the potential role of infectious diseases and HIV specialists in expanding PrEP implementation (Abstract 891). The researchers conducted a cross-sectional survey of 102 individuals seeking PrEP from the HIV clinic at Washington University in St Louis, in Missouri. The median age of this cohort was 29 years, 88% were MSM, 31% were black, and 70% reported engaging in anal sex without a condom in the past 3 months. Overall, 65% had a primary care practitioner (PCP), and 48% asked their PCP for PrEP, which was not prescribed. Thirty-nine percent of individuals seeking PrEP from their PCP reported feeling uncomfortable discussing sexual practices with their PCP. Sixty-one percent of those who reported feeling comfortable discussing sexual practices with their PCP asked for PrEP but were not prescribed it. As part of provision of PrEP, the physicians in the HIV clinic referred 83% of those seeking PrEP to a culturally sensitive PCP for primary care and continued provision of PrEP. The investigators concluded that PrEP can be a gateway to health care (as 35% had no PCP in this cohort) and that infectious diseases and HIV specialists can be a gateway to PrEP and can help link individuals to primary care facilities and practitioners. Further, the investigators proposed a PrEP-to-PCP implementation continuum in which infectious diseases and HIV specialists play leadership roles in developing and supporting a network of primary care clinics and practitioners to whom patients taking PrEP can be transitioned for ongoing PrEP and primary care.

As adherence is crucial for PrEP effectiveness, several researchers presented data on rates and correlates of adherence to PrEP and coverage of sex events by PrEP at CROI 2016. Marcus and colleagues reported results from a cohort study of 972 individuals who initiated PrEP in the Northern California Kaiser Permanente health care system (Abstract 894). The mean age at initiation of PrEP was 37 years, 98% were men, and 70% were white. Median adherence was 97% as measured by pharmacy refills, with only 3% with less than 60% adherence. Younger age, black or Hispanic race, and smoking were associated with low adherence ($P < .01$, for all). Overall, 219 (23%) individuals discontinued PrEP. In multivariate analyses, women (RR, 2.1; 95% CI, 1.4-3.2), recreational drug or alcohol use (RR, 1.6; 95% CI, 1.1-2.2), and a copay of more than $50 per month (RR, 1.4; 95% CI, 1.0-1.9) were independently associated with discontinuation of PrEP. There were no seroconversions among 850 person-years of individuals taking PrEP; however, 2 individuals seroconverted after discontinuing PrEP. These findings highlight the importance of developing strategies to support continued use of PrEP during periods of risk.

Holtz and colleagues reported on predictors of coverage of sex events among Thai MSM and transgender women taking PrEP in the ADAPT (Alternative Dosing to Augment PrEP Pill Taking) trial (Abstract 884). Participants in this study were randomly assigned to 1 of 3 self-administered dosing regimens for 24 weeks: daily, time driven (twice weekly with a postsex dose), or event driven (before and after sex). For all 3 arms, coverage was defined as having taken 1 or more pills in the 4 days before sex and 1 or more pills in the 24 hours after sex. Among 178 participants enrolled, the proportion of sex acts covered by PrEP was similar in the daily (85%) and time-driven (84%) arms, with fewer tablets required in the time-driven arm. In a multivariable model, age of 25 years to 35 years (compared with age of < 25 years), completion of college, and moderate or high reported alcohol use at baseline (as measured by Alcohol Use Disorders Identification Test [AUDIT] score) were associated with higher coverage of sex acts by PrEP, and baseline use of stimulant drugs and higher frequency of sex in the past 3 months were associated with lower coverage ($P \leq .05$, for all).

In the ADAPT trial, the proportion of sex events covered by PrEP was similar with daily versus time-driven dosing in Thai MSM and transgender women.

In the same study, Mannheimer and colleagues examined factors associated with coverage of sex events among 179 MSM and transgender women taking PrEP in the Harlem

neighborhood of New York City (Abstract 885). The median age was 30 years and 60% were black in this cohort. Coverage was highest among those receiving daily PrEP (66%), compared with time-driven (47%) and event-driven (52%) PrEP. Across both sites, incomplete coverage among those not taking PrEP daily was most often attributable to missing the postsex dose. In multivariate analyses, being in the daily dosing arm, older age, employment, and higher motivation to take PrEP (based on the information-motivation-behavioral Skills model) were associated with higher coverage by PrEP in the Harlem cohort, and black race and heroin use were associated with lower coverage ($P \leq .05$, for all). The investigators recommend further research to assess determinants of racial differences in coverage of sex events by PrEP.

Molina and colleagues presented data from the open-label phase of the IPERGAY trial of on-demand PrEP among MSM (Abstract 886). The randomized phase of this study evaluated a regimen of 2 pills of TDF and emtricitabine 2 hours to 24 hours before sex and 1 pill each 24 hours and 48 hours after sex, and demonstrated an 86% reduction in HIV infections in the group that received TDF and emtricitabine compared with placebo. Participants who were being followed or screened in IPERGAY were offered open-label TDF and emtricitabine and continued follow-up every 2 months. Among 362 participants enrolled, only 1 individual seroconverted during the open-label phase, resulting in an HIV incidence of 0.40 per 100 person-years (95% CI, 0.01-2.25 person-years), compared with 0.91 per 100 person-years and 6.6 per 100 person-years in those who received TDF and emtricitabine and placebo, respectively, in the double-blind phase. This participant did not elect to use PrEP in the open-label phase, had undetectable drug in plasma, and no drug resistance was detected. Overall, 33% acquired at least 1 STI during open-label follow-up. Median number of sex partners and episodes did not differ between the open-label and blinded phases of the study. Participants used a median of 18 pills per month—based on pill returns—although this may overestimate pill use, as participants were somewhat reluctant to return pills because TDF and emtricitabine was not available outside of the study. Few serious adverse events were reported (4% of participants), and only 1 participant discontinued PrEP because of elevated creatinine. Drug-related gastrointestinal events were reported in 10% of participants.

Sagaon-Teyssier and colleagues identified behavioral trajectories for use of PrEP and condoms over time in the blinded phase of the IPERGAY trial (Abstract 887). Among 332 participants who reported engaging in anal sex at least once during follow-up, 4 patterns of PrEP use were identified using a tailored methodologic framework: systematic use (high levels of PrEP use at last sex throughout follow-up) (40% of cohort); high-level progressive use (moderately high levels of PrEP use during follow-up) (31% of cohort); declining use (high levels initially, then declining over time) (13% of cohort); and low-level use (PrEP use low throughout follow-up) (16% of cohort). For condom use, 2 trajectories were identified: high-level use (70% of cohort) and low-level use (30% of cohort). Among those who reported low-level condom use, 23% reported declining or low-level use of PrEP, constituting the most at-risk group. This highest-risk group was more likely to be older (OR, 1.05; $P < .001$), have a lower level of education (OR, 1.91; $P = .02$), and to report sexual dissatisfaction (OR, 2.09; $P < .001$). The investigators concluded that although most MSM in IPERGAY used PrEP, condoms, or both during their most recent sexual episode, a substantial proportion did not use either and may benefit from additional prevention support.

Modeling the Impact of PrEP

Glaubias and colleagues modeled the potential impact of scaling up the dapivirine vaginal ring as PrEP in KwaZulu-Natal, South Africa (Abstract 1057). Prioritizing PrEP to 80% of sex workers was cost saving, and scaling up PrEP had greater preventive impact when focused toward women aged 20 years to 29 years (8% of infections averted, $3,309 per infection prevented) compared with women aged 15 years to 24 years (5.5% of infections averted, $5,209 per infection prevented).

Scaling up of PrEP with the vaginal ring decreased the prevalence of drug resistance, even when adherence was low.

The cost per infection averted decreased by more than half with increased adherence to PrEP (from 50% to 95%). Scaling up of PrEP decreased the prevalence of drug resistance, even when there was low adherence; however, these reductions in resistance diminished by 2% to 12% when resistance was tracked in both the blood and genital compartments. The investigators concluded that the dapivirine vaginal ring could have a substantial impact on HIV prevention at reasonable cost when prioritized by age, and could decrease drug resistance even at lower adherence levels.

Smith and colleagues presented modeling data on the cost-effectiveness of the dapivirine vaginal ring in South Africa (Abstract 1058). In a deterministic model in which the dapivirine ring was prioritized to female sex workers, young women, and those with more than 1 sexual partner and efficacy ranged from 25% to 75%, the dapivirine ring could prevent up to 13,000 new HIV infections per year on average and could be cost-effective if prioritized to those at greatest risk. Uniform coverage of the ring across risk groups had a larger impact due to high numbers of low-risk women being covered; however, this approach was more expensive than a more targeted strategy. The researchers concluded that the dapivirine ring could be cost-effective even in circumstances of low efficacy, and highlighted that the success of the ring is also affected by real-world user interest and adherence, which will be evaluated in upcoming open-label studies.

Yaylali and colleagues presented modeling data on the impact of improving HIV care and treatment and initiating PrEP in the United States (Abstract 1051). The researchers modeled

the impact of increasing HIV diagnoses, care, and treatment to the US National HIV/AIDS Strategy 2020 goals: 90% of infected individuals diagnosed, 85% of newly diagnosed individuals linked to care, and 80% of diagnosed individuals virally suppressed, and the marginal benefit of delivering PrEP to individuals at risk for HIV acquisition. In the base case, PrEP reduced new HIV infections by 18% over 5 years. Increasing diagnoses, care, and treatment to National HIV/AIDS Strategy 2020 goals had the largest impact on reducing new HIV infections (63%) in the United States. Although the marginal benefit of PrEP decreased as rates of viral load suppression increased, PrEP continued to achieve further reductions in HIV incidence, particularly among MSM.

Nichols and colleagues modeled the impact and cost-effectiveness of daily and on-demand PrEP among MSM in the Netherlands (Abstract 1052). Using a deterministic mathematical model of the HIV epidemic in the Netherlands, PrEP is targeted to 4500 MSM (approximately 2%-3% of all MSM in the Netherlands) with at least 1 new sexual partner per year. Over 12 years of PrEP scale-up and implementation, PrEP is predicted to avert between 1000 and 2500 (7%-13%) new HIV infections. The cost-effectiveness of PrEP increases with higher effectiveness of PrEP, decreased medication costs, and if the HIV epidemic remains stable (compared with the HIV epidemic declining as a result of HIV treatment scale-up). Under most scenarios, the use of PrEP is only cost-effective (< €20,000/quality-adjusted life-year gained) when used on demand. The authors recommended a price reduction of greater than 30% to ensure that on-demand PrEP remains cost-effective in the Netherlands, regardless of future declines in the epidemic.

Antibodies for HIV Prevention

In a plenary presentation, Mascola provided an overview of the use of passive immunization with antibodies for HIV prevention and treatment (Abstract 15). Antibodies have been used for prevention or early treatment of numerous viral infections, including the hepatitis A and B, varicella-zoster, rabies, and respiratory syncytial viruses. Since 2009, several potent neutralizing monoclonal antibodies (mAbs) against HIV-1 have been identified. These mAbs differ in potency and breadth of coverage of different HIV strains, and newer antibodies are up to 500-fold more potent than first-generation mAbs. Several preclinical studies of nonhuman primates have demonstrated the ability of neutralizing mAbs to protect against mucosal SHIV challenge when administered before or soon after viral exposure. mAbs to 4 major HIV binding sites are under development and several of these will be tested in clinical trials over the next few years. VRC01 is an mAb that attaches to a functionally conserved region of the CD4+ cell binding site, blocking viral entry, and neutralizes 80% to 90% of diverse viruses, regardless of clade. Preclinical studies have identified serum levels of VRC01 predicted to provide protection, and pharmacokinetic data from a phase I study suggests that protective levels of antibodies can be achieved with infusions every 2 months.

Key unanswered scientific questions include whether antibodies can prevent HIV infection in humans, what level of mAb is needed for protection, where and how mAbs work, and whether Fc receptor–mediated effector functions (antibody-dependent cell-mediated cytotoxicity) are needed for protection. Mascola described the phase IIb AMP (Antibody Mediated Prevention) study, conducted jointly by the HIV Vaccine Trials Network (HVTN) and the HPTN, which was designed to address a number of these questions. This trial will enroll 2700 MSM and transgender women in the Americas and 1500 women in Africa and is anticipated to launch in the second quarter of 2016. If VRC01 is found to be safe and effective in this proof-of-concept trial, it could pave the way for developing a subcutaneous injectable antibody product that could be given once every 3 months to 4 months for larger-scale use. Laboratory studies are underway to develop antibodies with greater potency and breadth, and strategies to combine antibodies could achieve higher levels of coverage. In addition, approaches to extend the half-life of antibodies are under investigation. In particular, mutations in the constant region of the antibody have resulted in prolonged circulating half-life, and a new version of VRC01 with this mutation is currently under study in a phase I trial.

The use of broadly neutralizing antibodies for HIV prevention is a promising approach to HIV prevention and will be evaluated in upcoming efficacy trials.

Mayer and colleagues presented data on the safety and pharmacokinetics of multiple doses and schedules of VRC01 in the HVTN 104 study (Abstract 90). In this trial, 88 low-risk men and women were randomly assigned to receive 1) a 40 mg/kg intravenous loading dose followed by 20 mg/kg intravenously every 4 weeks or 10 mg/kg, 30 mg/kg, or 40 mg/kg of VRC01 intravenously every 8 weeks; or 2) a 40 mg/kg intravenous loading dose followed by a 5 mg/kg subcutaneous dose of VRC01 or a placebo every 2 weeks. Infusions and injections were generally well tolerated in this study; mild pain or tenderness was observed in 28% of infusions and 14% of injections, and very few had erythema or induration. Overall, 57% of participants had at least 1 systemic symptom during the trial, most of which were mild; malaise and fatigue, headaches, and myalgia were the most common. For 76% of injections, no systemic symptoms were reported. Only 6% of adverse events were assessed as product related and all were mild and transient, and product was discontinued for 3 participants out of caution. After a 40 mg/kg intravenous loading dose, the mean VRC01 nadir level was 14 mcg/mL after 6 months of biweekly subcutaneous injections and 45 mcg/mL for 20 mg/kg VRC01 given intravenously monthly. For the regimens of 10 mg/kg, 30 mg/kg, and 40 mg/kg, peak concentrations were between 113 mcg/mL and 486 mcg/mL and nadirs were between 4 mcg/mL and 16 mcg/mL, with evidence of drug accumulation

after each dose. Trough concentrations for the 10 mg/kg and 30 mg/kg regimens were associated with high levels of coverage of clade B and C viruses in other studies of in vitro neutralizing activity. These safety and pharmacokinetic results support the use of these regimens in the upcoming phase IIb AMP study evaluating the efficacy of VRC01 for HIV prevention.

Conclusion

In summary, studies of HIV transmission and prevention were prominently featured at CROI 2016, providing insights into populations heavily impacted by HIV infection across the globe, and identifying gaps and potential strategies to address disparities in the epidemic. PrEP could have a substantial impact on HIV incidence, and data suggest increasing knowledge and uptake of PrEP in certain populations. Advances in novel PrEP drugs and formulations are promising but also raise potential implementation challenges. Additionally, harnessing antibodies for prevention is a novel, emerging approach currently under investigation.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

Financial affiliations in the past 12 months: Drs Buchbinder and Liu have participated in research trials that have received provision of medicines from Gilead Sciences, Inc.

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Invited Review

CROI 2016: Neurologic Complications of HIV Infection

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The brain remains a major target for HIV infection and a site of potential complications for HIV-infected individuals. Emerging data presented at the 2016 Conference on Retroviruses and Opportunistic Infections suggest that during the early stages of infection, activated CD4+ cells may traffic the virus into the central nervous system (CNS). HIV is detectable in cells and tissues of the CNS in some individuals despite suppressive antiretroviral treatment. A potential source of cerebrospinal fluid HIV escape may be compartmentalized HIV replication within macrophage lineage cells. Virally infected cells can traffic out of the CNS and may have the potential to reseed the systemic compartment. Additional modifiers of HIV-associated neurocognitive disorder (HAND) were identified, including female sex and hepatic dysfunction. Large epidemiologic studies reported an elevated risk of stroke among HIV-infected individuals, related to traditional vascular risk factors, history of recreational drug use, and HIV measures (lower CD4+ cell nadir and higher viral load). Brain imaging may provide a noninvasive means for detecting early changes in the brain associated with HIV infection and may assist in prognosis of HAND. Some potential adjunctive therapies to standard antiretroviral therapy for HIV-infected individuals were considered.

Keywords: CROI, 2016, HIV, central nervous system, HIV-associated neurocognitive disorder, neuroimaging, neuropathogenesis, HIV reservoirs, stroke, cerebrospinal fluid

The status of the central nervous system (CNS) in HIV-infected persons was a central theme in several sessions at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI). One symposium session (Session S-2,"A Beautiful Mind: Keeping It") highlighted some of the most important themes of CNS HIV infection, including cerebrospinal fluid (CSF) and blood biomarkers of CNS HIV involvement (Abstract 60), state-of-the-art tools and applications for neuroimaging (Abstract 61), establishment and maintenance of the CNS as a site of HIV persistence (Abstract 62), and challenges and approaches for treatment of CNS complications (Abstract 63). Further, CNS-related themes emerged at the conference outside of neurologic-specific sessions, including sessions focused on pediatric HIV infection, basic science investigations, vascular complications, and tissue HIV reservoirs. Neurologic presentations focused on themes relevant to persistent CNS dysfunction in the treated HIV-infected individual: the CNS as a potential reservoir for HIV, including initiation and persistence of HIV infection in the CNS; contributors to clinical manifestations of CNS HIV; cerebrovascular disease in HIV infection; and neuroimaging tools for detection of CNS abnormalities. Finally, assessment of potential treatments for HIV-associated neurocognitive disorder (HAND) yielded several promising approaches and set the stage for further in-depth studies of therapeutic strategies to address persistent abnormalities affecting some individuals despite well-treated HIV infection.

The CNS as a Site of HIV Persistence: A Barrier to Cure?

The potential of HIV cure—either achieving complete viral eradication or effecting long-term HIV remission in the absence of antiretroviral treatment—has stimulated intense interest in whether tissues and cells outside of the systemic compartment and lymph nodes may be meaningful sites of HIV persistence during therapy. In a symposium talk, Swanstrom (Abstract 62) provided an overview of the concept of the CNS as an HIV reservoir, describing the emergence of CNS compartmentalization of HIV before the initiation of antiretroviral therapy, cases of viral escape with evidence of HIV replication in the CNS despite systemically suppressive therapy, and the evolution of macrophage-tropic HIV Env presumed to facilitate productive infection of resident CNS macrophages and microglial cells.

Numerous talks and posters at the conference focused on specific topics introduced in Swanstrom's overview. Stefic and colleagues (Abstract 400) investigated the mechanisms of CNS compartmentalization of HIV among 9 participants with previous exposure to antiretroviral medications, low CD4+ cell counts (median 163/μL), and HAND. Using single-genome amplification of env from paired blood and CSF samples, the investigators detected compartmentalization in 55% of participants. Compartmentalized HIV had a greater degree of diversity between compartments, and sequencing revealed CSF-specific amino acid signatures, some previously reported and some newly described, in the env gene across several subtypes of HIV. The sensitivity to autologous neutralizing antibodies of pseudotyped HIV did not differ among samples derived from blood and those derived from CSF. In contrast, sensitivity to purified broadly neutralizing antibodies differed substantially in many cases between blood and CSF. However, patterns of sensitivity to neutralizing antibodies of

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either type did not relate to viral compartmentalization of CSF. These data suggest that CNS compartmentalization of HIV may chiefly derive from factors outside of selective pressures of autologous neutralizing antibodies, and may relate instead to genetic attributes related to cell entry.

Seipone and colleagues (Abstract 398) approached the question of whether a concurrent opportunistic infection in the CNS might impact the extent of HIV compartmentalization in the CNS by assessing HIV replication among HIV-infected individuals with or without documented tuberculous meningitis (TB meningitis). The investigators found statistically significantly higher levels of HIV RNA in the CSF in 15 individuals with TB meningitis as compared to 22 individuals without TB meningitis. However, in an analysis of env sequencing by single-genome amplification that compared degree of compartmentalization between 4 participants with TB meningitis and 4 without, no clear patterns emerged. This study was limited by a small sample size, but the finding of higher HIV RNA levels in CSF in individuals with an opportunistic infection in the CNS warrants further analysis, specifically of the interaction between HIV replication dynamics and concurrent inflammatory disorders in the CNS, including opportunistic infections.

In a complementary longitudinal study, Bowman and colleagues (Abstract 401) confirmed a relationship between HIV compartmentalization in the CNS and neurocognitive response to antiretroviral therapy. Using single-genome amplification or deep sequencing of HIV env in CSF and blood, the investigators detected HIV compartmentalization in the CNS in 35% of 28 study participants before the initiation of antiretroviral therapy at CD4+ cell counts below 400 copies/μL. The effect of HIV compartmentalization was examined with respect to performance on a detailed neuropsychologic testing battery at baseline and at 6 months and 12 months after starting treatment. At the baseline visit, no laboratory parameters differed between the compartmentalized and noncompartmentalized groups, and neurocognitive impairment was not statistically significantly higher in the group with compartmentalization than in the group without. However, at 6 months and 12 months after initiation of antiretroviral treatment, the overall global deficit score, a measure of neurocognitive impairment, was statistically significantly lower in the noncompartmentalized than in the compartmentalized group. An interpretation of this finding may be that measurable HIV compartmentalization in the CNS before the initiation of antiretroviral therapy reflects a robust site of HIV replication in the CNS that is less responsive to therapy due to reduced antiretroviral exposure in CNS tissues or cells of replication. Additionally, or alternately, compartmentalized HIV replication in the CNS before antiretroviral treatment may result in more severe inflammatory and neural injuries that are irreversible or slower to reverse with therapy.

In a related analysis, Evering and colleagues (Abstract 406) used single-genome amplification to assess the relationship between drug resistance mutations in blood and CSF and the presence of HAND in 12 participants with virologic failure during antiretroviral therapy in the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) study. Five participants had normal neurocognitive function and 7 had HAND. The presence of drug resistance mutations in CSF and blood was statistically significantly higher among individuals with HAND, and compartmentalization of HIV-1 pol in the CNS was more frequent among individuals with HAND. Moreover, 43% of the participants with HAND had drug resistance mutations detected in CSF that were not detected in the blood. These findings may contribute to an understanding of the pathogenesis of HAND and, particularly, the development of viral escape in CSF among individuals without prolonged effective plasma viral suppression.

To better define the virologic basis of CSF viral escape, Joseph and colleagues (Abstract 402) described the virologic features of HIV env derived from individuals with asymptomatic CSF viral escape identified in a cohort study of 96 individuals receiving more than 1 year of antiretroviral therapy with plasma viral suppression. Six of these individuals were identified as having asymptomatic CSF viral escape. Of these 6 individuals, 2 had longitudinal sampling that revealed resolution of viral escape at 9 months, with 1 participant having continued persistence of viral escape (HIV RNA level 356 copies/mL in CSF, with undetectable plasma HIV RNA) at 8 months. Single-genome amplification sequencing of HIV env from CSF-derived samples in the participant with transient viral escape revealed a purely T-cell tropic, clonally expanded population. Similar examination of samples with persistent CSF viral escape revealed a genetically diverse HIV population with enhanced ability to infect cells with low CD4 receptor density, suggesting adaptation to a macrophage-tropic virus. These data suggest that HIV detected in CSF in individuals receiving systemically suppressive antiretroviral treatment can in some cases reflect low-level viral replication within macrophages or microglial cells.

Although macrophages and microglial cells are the principal sites of HIV replication within the CNS in HIV encephalitis, the possibility that HIV may infect other cell types within the CNS remains a focus of intense investigation. Astrocytes in particular have been controversial as a potential target for HIV, as they lack CD4 surface receptors. Li and colleagues (Abstract 393) investigated possible mechanisms of astrocyte HIV infection in humans, finding that infection by mature HIV virions could be facilitated ex vivo by transfecting astrocytes with plasmid encoding the CD4 receptor, by a use of a lysosomotropic agent, or by exposure to Tat peptide. Additionally, these investigators exposed astrocytes to newly produced HIV particles produced by infected CD4+ lymphocytes using a transwell culture pore system and found that after 3 weeks to 4 weeks, the HIV p24 antigen could be measured from culture media around the astrocytes. Anti–CXC chemokine receptor 4 antibodies but not anti-CD4 antibodies inhibited infection. These data that distinguish between mechanisms of infection of astrocytes by mature and newly produced HIV set the stage for future important investigation of the possible conditions for restricted HIV infection of this cell type.

The existence of a viral reservoir is defined not only by the potential for HIV infection of a given cell or tissue but also by

the persistence of HIV in the setting of apparently effective antiretroviral treatment. de Oliveira and colleagues (Abstract 143) assessed paired blood and CSF samples from 16 participants with suppressed HIV RNA in blood at a median 2 years after initiation of early (before 4 months of estimated infection) or delayed (after 14 months of estimated infection) antiretroviral therapy. Cell-associated HIV DNA in peripheral blood mononuclear cells (PBMCs) and CSF cellular pellets was measured by digital droplet polymerase chain reaction (PCR) testing, followed by next-generation sequencing of partial env from these samples and subsequent phylogenetic analysis. HIV DNA was detected overall in 15 of 16 PBMC samples and 10 of 16 CSF samples. Although CSF interleukin (IL)-6 and tumor necrosis factor–α were lower among those in the early treatment group, there was no difference between HIV DNA detectability in either sample type between the 2 treatment groups. Genetic analysis of env sequences from 8 paired CSF and blood samples revealed that 7 had statistically significant HIV compartmentalization in the CNS, with unique CSF-specific sequences detected despite early treatment and longitudinal persistence of CSF-unique sequences over a 5-month period during treatment. A challenge of these experiments is the low HIV DNA input from the CSF samples, which could bias compartmentalization analysis. Overall, the findings are consistent with recognition of compartmentalization in the CNS early in untreated HIV infection and suggest the potential persistence of compartmentalized HIV in cells of the CNS despite treatment.

Lamers and colleagues (Abstract 345) similarly demonstrated that HIV DNA persisted in the CNS compartment along with other tissue sites among 20 individuals with undetectable HIV RNA (using a lower limit of detection of 40-400 copies/mL) in plasma at autopsy. Fifteen of these individuals had cancer, and the majority were documented as taking antiretroviral treatment at the time of death, with the remainder documented as taking antiretroviral treatment near the time of death. HIV DNA was detected by digital droplet PCR assay in the majority of 87 brain tissue samples analyzed. Nearly all brain tissue samples demonstrated pathology, although only a few showed classic HIV-associated changes of microglial nodule encephalitis or CD68+ infiltrates. An RNAscope assay detected HIV RNA colocalizing with CD68+ cells, likely macrophages, in brain samples from 2 donors with CNS malignancies, and single-genome sequencing revealed clustering of HIV DNA from brain with other anatomic compartments. These data are some of the first to demonstrate the persistence of HIV DNA and even HIV RNA in the brains of infected individuals taking apparently systemically successful antiretroviral treatment, necessitating further research into potential HIV persistence in the CNS despite suppression of high-level HIV replication.

Emerging data indicate that HIV DNA and RNA may continue to be detectable in cells and tissues of the CNS in some individuals taking suppressive antiretroviral treatment. A potential source of escape of HIV in CSF may be compartmentalized HIV replication within macrophage lineage cells.

CNS HIV Entry and Immune Cell Trafficking

A complete understanding of the mechanisms that establish and maintain CNS HIV infection is key to designing interventions to address potential HIV reservoirs in the CNS. HIV enters the CNS in the first weeks after transmission to the host and can be detected throughout the course of untreated HIV infection. However, the means by which HIV is trafficked into the CNS compartment during initial and chronic infection is incompletely understood. A number of studies focused on aspects of trafficking of HIV or immune cells in or out of the CNS during the course of infection.

In an effort to examine determinants of the level of HIV present in the CNS in the earliest stages of infection, Schuetz and colleagues (Abstract 404) examined various sites, including gut mucosa, PBMCs, and CSF in 38 participants in Thailand with antibody-negative acute HIV infection at an estimated duration of infection of 15 days. The investigators found correlations between measures of immune activation (percentage of CD8+ and Ki67+ cells) in the blood and gut and the level of HIV RNA in the CSF, independent of the level of plasma HIV RNA. These data support the hypothesis that peripheral immune activation may facilitate entry of HIV-infected cells into the CNS compartment, mediating the relationship between HIV RNA produced in the periphery and that detected in the CSF.

Two studies examined the relationship between immune cells in blood and CSF during the early stages of HIV infection and the extent of viral trafficking to the CNS. Trautmann and colleagues (Abstract 407) characterized CD8+ cells from the CSF of 28 individuals with acute HIV infection from the same cohort in Thailand. The investigators demonstrated the emergence of high levels of CD8+ cell activation (defined as the percentage of CD38+ and HLA-DR+ cells among all CD8+ cells) in CSF during Fiebig stage II or III HIV infection. The percentage of activated CD8+ cells in CSF correlated with levels of HIV RNA in CSF across Fiebig stages, supporting the concept that immune activation in the CNS is associated with RNA production. A high proportion of CD8+ cells in CSF were HIV-specific cells, and these cells manifested patterns of V beta families distinct from those in blood, suggesting unique T-cell repertoires. These results suggest that even during the initial stages of HIV infection, distinct T-cell responses characterize the CNS and may reflect and facilitate compartmentalization of immune response and perhaps viral infection.

Li and colleagues (Abstract 142) examined CD8+ and CD4+ cells and monocytes in paired CSF and blood samples obtained in a longitudinal study of men recently infected with HIV. Beginning at a median 3.3 months post infection, the percentage of activated CD4+ and CD8+ cells increased at an accelerated rate in the CNS compartment compared with the blood during untreated infection; percentage of activated cells in the CNS compartment did not measurably decline

during 7 months of follow-up during antiretroviral therapy, despite declining in the blood. Moreover, HIV RNA concentrations in the CSF independently correlated with the percentage of activated CD4+ cells but not of CD8+ cells or activated or trafficked monocytes in CSF. These findings suggest that during the early stages of infection, activated CD4+ cells trafficking to the CNS might be a major source of HIV replication, modifying the traditional concept that infected monocytes are the primary "Trojan horse" carrying HIV to the CNS (for review, see Abstract 62).

The concept that CD4+ T lymphocytes may be a key cell type trafficking to the CNS early in the course of infection was supported in a presentation by Vasan and colleagues (Abstract 405) regarding CNS findings after acute infection in a nonaccelerated simian-human immunodeficiency virus (SHIV) model. This model closely resembles human disease in terms of systemic viral load and immune responses, as well as CSF biomarker patterns. On examination of tissues, CD8+ cellular infiltrates were noted to be surrounding blood vessels in the brain, and CD4+ cells were found clustered in the meninges of SHIV-infected macaques at 12 weeks after infection but not in uninfected animals. These CD4+ cells aggregating in the meninges might be a CNS-specific source of SHIV production in the early stages of infection, contrasting with macrophage and microglial cell sources of viral RNA present in established infection and encephalitis.

In a study focused on a macaque model of late-stage simian immunodeficiency virus (SIV) disease, Mallard and colleagues (Abstract 403) found shared SIV gp120 sequences to be present between monocytes and macrophages detected in the bone marrow and the brain of CD8-depleted animals with SIV encephalitis. Analysis using Bayesian evolutionary analysis sampling trees (BEAST) to determine time to most recent common ancestor revealed recent viral spread between bone marrow and brain, suggesting a bone marrow source of virally infected cells in the CNS during later stages of infection.

Although many presentations centered on ingress of cells and HIV into the CNS, the possibility that cells originating in the CNS may traffic out of this compartment into the periphery is a concern of key importance to HIV eradication efforts. If HIV-infected immune cells or free virus might egress from the CNS, then persistent infection in CNS cells may lead to reseeding of HIV in the systemic compartment despite successful systemic HIV remission or eradication. Recent evidence that the brain has a dedicated lymphatic system that allows for trafficking of immune cells directly from the CNS to the deep cervical lymph nodes supports this concept,[1] but trafficking of infected cells has not previously been observed.

Alvarez and colleagues (Abstract 141) presented evidence that infected cells traffic from the CNS, employing superparamagnetic iron oxide nanoparticles (SPIONs; foreign particles likely to be ingested by phagocytic cells, including macrophages and microglial cells in the brain). The investigators injected fluorescent SPIONs into the cisterna magna of the brain of SIV-infected or uninfected macaques, monitoring SPION uptake by perivascular cells during the first day after injection. Robust transit of SPION-containing cells from the CNS to the cervical lymph nodes was observed over the following 7 days in both infected and uninfected animals, and these cells were characterized as CD163+ cells, indicating a macrophage-monocyte lineage. With staining, the investigators confirmed that some of the SPION-containing cells in the lymph nodes of infected animals were SIV infected, demonstrating that in this accelerated macaque model, infected cells were trafficked from the CNS to the cervical lymph node. These data suggest a route of viral infection from the CNS to the periphery, highlighting the need to adequately treat the CNS compartment during standard HIV treatment and to consider this compartment in viral eradication efforts.

The ability to address immune abnormalities that persist in the CNS in the context of suppressive antiretroviral therapy will be essential to optimal treatment of HIV infection. HIV RNA levels, soluble measures of inflammation, and CSF cell characteristics improve in response to systemically suppressive antiretroviral therapy but may not completely normalize despite prolonged treatment.[2,3] Chung and colleagues (Abstract 411) demonstrated the feasibility of applying both flow cytometry and newly emerging mass spectrometry methods to examine characteristics of CSF and blood cells in HIV-infected individuals well treated with antiretroviral medications, despite having low clinical CSF white blood cell counts. Mass spectrometry, unlike flow cytometry, uses heavy metals rather than fluorescein dyes to label cell surface receptors, allowing for resolution of up to 40 distinct surface markers simultaneously in a single sample. Among 7 individuals taking antiretroviral treatment, with a median white blood cell count of 2/μL, mass spectrometry generated interpretable data and identified a unique subset of effector memory cells comprising the majority of T cells in CSF but not blood. These methods have potential in future investigations of individuals with treated HIV infection, as a means of identifying unique abnormalities that should be mitigated in the optimal treatment of HIV infection in the CNS.

A detailed understanding of HIV and immune cell trafficking is emerging from studies using human and animal models of acute and chronic HIV infection, suggesting a role of activated T lymphocytes and macrophages that migrate into and out of the CNS.

Contributors to Presentation and Progression of HAND

A key concern for clinicians and persons living with HIV infection is HAND, a condition that can impact individuals despite treatment with antiretroviral therapy. Numerous studies focused on potential factors that may contribute to HAND. Potential differences between HAND in women and in men were explored by Maki and colleagues (Abstract 416), through a comparison of cognitive test performance among women enrolled in the WIHS (Women's Interagency HIV Study)

study and men enrolled in the MACS (Multicenter AIDS Cohort Study) (429 HIV-infected and 281 uninfected individuals in each group). The investigators compared performance on 4 common neuropsychologic tests utilized in both studies, using a mixed-effects regression analysis that matched participants by demographic and disease variables. HIV-infected women performed statistically significantly worse on Trail Making and Grooved Pegboard tests, tests of executive function and processing speed, than HIV-infected men. Differences between women and men in these measures may reflect biologic or sociodemographic differences, but this study provides rationale to further investigate the sources of these differences and whether distinct interventions may be required to address them.

Valcour and colleagues (Abstract 422) performed a novel exploration of the potential contribution of minimal hepatic encephalopathy associated with liver fibrosis and HAND among women in the WIHS study. Neuropsychologic testing results among 258 women with liver fibrosis—defined by aspartate aminotransferase (AST)-to-platelet ratio index—were compared with those of 1221 women without liver fibrosis within the WIHS study. After adjustment for hepatitis C virus (HCV) infection status and HIV disease markers, the presence of liver fibrosis was associated with poorer overall cognition and performance on verbal learning, executive function, verbal memory, psychomotor speed, fluency, and fine motor tasks. HCV infection status had no independent association with cognition. These findings suggest that liver fibrosis may be an additional contributor to cognitive dysfunction in HIV-infected women in particular and possibly in persons living with HIV infection more generally. These results suggest possible new specific therapeutic interventions for HAND in individuals who have concomitant hepatic dysfunction.

In another assessment of potential toxic metabolic contributors to HAND, mitochondrial dysfunction and neurocognitive performance were evaluated by Samuels and colleagues (Abstract 144), in a cohort of 1011 participants from the CHARTER study. This study revealed a relationship between mitochondrial DNA content in blood cells and the presence of cognitive impairment. This association was primarily driven by correlations among individuals with only incidental comorbidities, suggesting that mitochondrial DNA content in blood cells may be a contributor to HAND in the absence of other factors. In a subset of 335 study participants, elevated cell-free mitochondrial DNA in the CSF was associated with increased measures of HIV RNA, inflammation, and iron metabolism in the CSF but not with neurocognitive performance.

Hellmuth and colleagues explored the neurologic manifestations (Abstract 415) and psychiatric symptoms (Abstract 414) accompanying acute HIV infection in individuals identified with very early antibody-negative infection in Bangkok, Thailand. Mild neurologic signs, including slowed fine finger movements and neuropathy, and mild symptoms of cognitive difficulties affected the majority of individuals during very early infection. However, in this cohort that received antiretroviral treatment during acute infection, the majority of findings remitted during 3 months of follow-up. Self-reported mood difficulties, including depression and anxiety, were also highly prevalent at baseline in this cohort and statistically significantly correlated with HIV disease indices, including lower CD4+ cell counts in blood and higher levels of HIV RNA and neopterin (a macrophage activation marker) in blood or CSF. Prevalence of depression and anxiety decreased dramatically after 12 weeks of antiretroviral treatment in this cohort. These findings suggest that although abnormal neurologic and psychologic findings may manifest extremely early in HIV infection, early initiation of treatment may help to ameliorate processes that contribute to HAND in chronic HIV infection.

Perhaps consistent with this hypothesis is a report from Vassallo and colleagues (Abstract 408) who investigated the longitudinal relationship between CD4+/CD8+ cell ratio in blood and neurocognitive dysfunction over approximately 2 years among 96 participants in the Neuradapt study, a prospective study of HAND. The investigators found that in this cohort, in which 73% of individuals had plasma HIV RNA suppressed to less than 200 copies/mL, a decline in CD4+/CD8+ cell ratio was associated with a decline in performance on neurocognitive testing in a multivariable model (odds ratio, 3.70; $P = .007$). As the CD4+/CD8+ cell ratio reflects both immune suppression and excess systemic immune activation, this measure serves as a more complex index of immune dysfunction of persons living with HIV infection than CD4+ cell count or CD4+ cell count nadir alone. Although mechanisms driving the decline or recovery of the CD4+/CD8+ cell ratio are still largely unknown, early initiation of antiretroviral treatment leads to relative preservation of the CD4+/CD8+ cell ratio, potentially protecting the CNS from immunologic factors that contribute to HAND.

Mukerji and colleagues (Abstract 145) examined longitudinal associations between neuropsychologic testing results and both blood lipid parameters and the presence of an apolipoprotein E4 (APOE4) allele in men enrolled in the MACS study. The investigators examined trajectories of neurocognitive performance among 273 men with HIV infection who were taking antiretroviral therapy (aged 50-65 years) and among 516 matched uninfected men (aged 50-65 years). Among the HIV-infected men, but not uninfected men, abnormal lipid profiles (ie, elevated total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels, and reduced high-density lipoprotein level) were associated with accelerated declines in neuropsychologic testing performance in this age span. Similarly, the presence of an APOE4 allele was associated with a pattern of enhanced decline among HIV-infected men compared with uninfected men in the same age range. These findings suggest a possible important role of abnormal lipid metabolism in the development of neurocognitive impairment among middle-aged HIV-infected men taking HIV treatment. As statin use was not controlled for in the analysis, it is possible that individuals with lower-range lipid measurements had disproportionately higher statin use, which might benefit neurocognition in the context of HIV infection through lipid-independent antiinflammatory effects. However, it is also plausible that lipid metabolism impacts HAND by increasing the risk of vascular dysfunction that was

previously tied to the presence of HAND in the MACS and other cohorts,[4,5] providing a rationale for use of statins or other lipid-lowering agents in persons with HAND.

Stroke and Cerebrovascular Disease in HIV: An Emerging Concern

As HIV becomes more of a chronic disease, additional cofactors (eg, cerebrovascular disease) are becoming increasingly important when treating HIV-infected individuals.[6] Becker and colleagues (Abstract 388) suggested that cardiovascular disease risk factors may be as important as HIV-associated factors in predicting rate of change in the brain. Although the overall incidence and mortality of stroke among HIV-infected individuals has decreased, Berenguer and colleagues (Abstract 639) demonstrated that the risk of stroke still remains elevated among HIV-infected individuals compared with uninfected individuals, based on large epidemiologic studies. Even after controlling for traditional risk factors, HIV-infected individuals have a substantially higher risk of ischemic stroke than uninfected individuals.[7] Crane and colleagues (Abstract 636) showed that most strokes seen among HIV-infected individuals are ischemic rather than hemorrhagic in nature.

The overall incidence of ischemic stroke was 1.69% among HIV-infected individuals according to data presented by Chow and colleagues (Abstract 43). Although the risk of ischemic stroke is elevated across the lifespan of an individual, the greatest risk was actually seen in younger HIV-infected individuals. In particular, Chow and colleagues demonstrated that HIV-infected women have a higher risk of stroke even after adjustment for age, race, vascular, and sex-specific risk factors (Abstracts 638 and 43). The greatest risk of stroke among HIV-infected women occurred among those aged 40 years to 49 years. Chow and colleagues (Abstract 43) and Crane and colleagues (Abstract 636) identified traditional risk factors associated with ischemic stroke, including older age, elevated blood pressure, active or past recreational drug use, current smoking, diabetes, and HIV-associated variables (lower CD4+ cell nadir and higher viral load). The increased risk of ischemic stroke associated with unsuppressed virus corresponded to an effect of aging by approximately 15 years.

Hatleberg and colleagues (Abstract 637) identified risk factors for hemorrhagic stroke, including elevated blood pressure and poor renal function. Controversy still remains concerning the impact of HCV infection, as one study demonstrated an increased risk for hemorrhagic stroke among HIV/HCV-coinfected individuals. Although Berenguer and colleagues (Abstract 639) identified an increased risk of stroke among a large cohort of HIV/HCV-coinfected individuals in Spain, Crane and colleagues (Abstract 636) and Chow and colleagues (Abstract 43) did not observe a substantially increased risk among participants with HIV/HCV coinfection compared with those with HIV monoinfection in studies performed in the United States. For all epidemiologic studies, careful analysis and adjudication are needed, as the diagnosis and medical coding of stroke are often complicated and may be overrepresented (approximately 45% of strokes coded using the International Classification of Diseases [ICD]-9 required additional discussion by clinicians). As noted by Crane and colleagues (Abstract 636) and Chow and colleagues (Abstract 43), additional limitations of these large observational datasets include that information is not collected at regular intervals using standard metrics and that, typically, homogenous convenience samples are utilized. These cohorts often have a higher proportion of white men.

The risk of stroke remained elevated among HIV-infected individuals compared with uninfected individuals, based on large epidemiologic studies. Traditional vascular and HIV disease–related risk factors could be used to specifically target interventions toward high-risk HIV-infected populations.

Neuroimaging studies may assist in evaluating the effects of cerebrovascular disease and in visualizing preclinical changes, as noted by Becker and colleagues (Abstract 388). Janjua and colleagues (Abstract 640) noted increases in incidental carotid plaque (calcified and noncalcified) among HIV-infected individuals who were free of known cardiovascular disease compared with matched uninfected controls. In addition, the presence of carotid plaque was associated with an increased incidence of subsequent cerebrovascular events.

Stroke may be underreported as a distinct end point in many clinical trials involving HIV-infected individuals. Certain groups of HIV-infected individuals (eg, women or those of black race) may be at increased risk for stroke. These groups may merit specific targeting for possible interventions. Within the HIV-infected population, traditional (eg, hypertension, hyperlipidemia, diabetes, smoking, etc) and disease-related (eg, detectable HIV RNA or presence of immune activation) risk factors could be specifically targeted for future interventions.

Neuroimaging to Diagnose and Assess the Mechanisms of HAND

There is an expanding interest in using neuroimaging methods to study the effects of HIV infection on brain structure and function. Brain imaging may detect HIV-associated changes soon after initial infection.[8,9] In a cohort of HIV-infected individuals in Thailand, structural brain volumetric and metabolite measurements were performed by Killianpur and colleagues (Abstract 384) at diagnosis of acute HIV infection (< 1 month after seroconversion) and at 2-year follow-up. A 2% to 3% rate of atrophy was observed primarily in subcortical areas (caudate, putamen, and globus pallidus). Decreases in subcortical brain volumetrics correlated with increases in neuronal loss and inflammation (as measured by magnetic resonance spectroscopy). Slowly evolving, multidimensional changes may continue to progress if HIV-infected individuals remain untreated (Abstract 63). A number of groups, including Guha and colleagues (Abstract 382) and Cysique and colleagues (Abstract 391), demonstrated that continued active

viral replication and inflammation are often associated with subcortical changes. In particular, continued presence of virus was associated with reduced putamen volume in an analysis by Guha and colleagues (Abstract 382). With regard to progressive immunosuppression, Schonfeld and colleagues (Abstract 383) demonstrated that a lower CD4+ cell count nadir was associated with volumetric loss in cortical areas (including frontal, temporal, and parietal lobes).

The mechanism by which pathologic spread occurs from subcortical to cortical areas remains unknown, but an interesting study investigated the neurovascular unit. De Alwis and colleagues (Abstract 389) observed that HIV pathology caused intracranial vessel wall thinning and loss of vascular plasticity. This can cause an expansion of the vessel lumen and poorer regulation of perfusion to subcortical and cortical brain regions. Noninvasive imaging of changes in the arterial wall could potentially serve as an in vivo marker for monitoring disease progression and evaluating the potential benefits or deleterious effects of antiretroviral therapy in smaller vessels in HIV-infected individuals.[10]

Neuroimaging may provide a noninvasive means to evaluate progressive changes in the brain caused by HIV infection. Changes may initially occur within subcortical brain regions and spread to cortical areas with more advanced disease.

A larger number of neuroimaging studies have also begun to focus on the effects of HIV infection in pediatric populations. Hoare and colleagues (Abstract 821) showed that HIV-infected children performed statistically significantly worse on neuropsychologic performance tests in various domains (ie, processing speed, memory, language, and flexibility) than well-matched uninfected children. These HIV-infected children also had substantial abnormalities in brain structure, especially within the corpus callosum, compared with uninfected children. In addition, HIV-infected children whose initial antiretroviral regimen failed had greater white matter brain dysfunction.

In another series of studies, a cohort of youths with perinatally acquired HIV infection who were taking antiretroviral therapy had both cortical (as demonstrated by Williams and colleagues, Abstract 822) and subcortical (as demonstrated by de los Angeles and colleagues, Abstract 823) structural changes in the brain compared with uninfected youths in a matched cohort. The greatest decreases in cortical and subcortical volumetrics were associated with higher peak plasma viral loads and unsuppressed virus. In particular, de los Angeles and colleagues (Abstract 823) showed that subcortical (putamen, globus pallidus, caudate nucleus, and thalamus) structural changes were correlated with poorer scores on neuropsychologic performance testing. Williams and colleagues (Abstract 822) showed that alcohol and marijuana use were also linked to lower brain volumes, suggesting that not only HIV infection but other factors may influence brain development in HIV-infected youths.

Initiation of antiretroviral therapy leads to improvements in brain function. Schiffito (Abstract 392) reported encouraging results that 12 weeks after initiation of antiretroviral therapy, statistically significant improvements in functional connections were observed between the posterior cingulate cortex and other brain regions among previously treatment-naive HIV-infected individuals. Ances (Abstract 61) and Calcagno (Abstract 63) suggested that initiation of antiretroviral therapy soon after seroconversion may be most beneficial, although treatment does not lead to a complete normalization. High variability exists in penetration of the CNS. Numerous clinical and demographic factors may also affect concentrations of medications. Calcagno (Abstract 63) showed that HIV-infected individuals with well-controlled virus may still have residual HIV replication or residual systemic and CNS immune activation. Questions still remain concerning the optimal antiretroviral regimen and the best means to assess the effects of treatment (eg, CNS penetration effectiveness or monocyte efficacy score).

Overall, use of antiretroviral therapy has led to a reduction in the incidence but not prevalence of more severe forms of HAND.[11,12] Findings continue to suggest that despite the introduction of antiretroviral therapy and subsequent virologic control, there appears to be a substantial percentage of HIV-infected individuals who still have evidence of poorer cognitive performance, atrophy of grey and white matter, and abnormalities in white matter (Abstract 61). Underwood and colleagues (Abstract 148) used a k-means cluster method and found that brain and cognitive abnormalities often occurred together in HIV-infected individuals. In particular, increased atrophy of grey matter was associated with lower fractional anisotropy as measured by diffusion tensor imaging. Cysique and colleagues (Abstract 391) showed that HIV-infected individuals with a history of neurocognitive impairment often have loss of neuronal integrity within subcortical and cortical regions. Granziera and colleagues (Abstract 381) showed that volumetric changes in the brain may have longitudinal predictive power to detect subsequent changes in neuropsychologic performance. A combination of methods (neuroimaging, CSF analysis, and neuropsychologic performance testing) may therefore provide a more complete understanding of changes in the brain caused by HIV infection (Abstract 61). As noted by many of these studies, additional investigations are needed that 1) pool data from various modalities and cohorts (Abstracts 383 and 386); 2) longitudinally access HIV-infected individuals (especially those with well-controlled virus) (Abstracts 384 and 381); and 3) include appropriate uninfected controls for comparison (Abstracts 61 and 146).

A combination of methods (neuroimaging, cerebrospinal fluid analysis, and neuropsychologic performance testing) may provide a more complete understanding of brain changes caused by HIV infection.

Therapeutics for HAND

A major priority for HIV research efforts is the development of effective treatment strategies to improve neurocognitive disorders or to prevent the development of HAND. Several strategies were evaluated as adjunctive therapies to standard antiretroviral therapy. Most interventions, although promising in concept, did not have a measurable impact on clinical or laboratory outcomes. CC chemokine receptor R5 (CCR5) inhibitors have potential antiinflammatory and antileukocyte trafficking properties that may reduce immune activation and infection in the CNS. Winston and colleagues (Abstract 423LB) presented results from a study comparing neurologic outcomes in antiretroviral therapy–naive participants randomly assigned to initiate a protease inhibitor–based regimen of tenofovir disoproxil fumarate (TDF), emtricitabine, and ritonavir-boosted atazanavir or a protease inhibitor–based regimen of abacavir, lamivudine, ritonavir-boosted darunavir, and the CCR5 inhibitor maraviroc. Thirty participants were randomly assigned to each arm and followed up for 48 weeks; all participants achieved plasma viral suppression at follow-up. Both groups experienced improvement in neurocognitive performance, with no statistical differences detected between the groups.

Data from the large AIDS Clinical Trails Group (ACTG) A5303 study, which focused on the possibility that maraviroc might specifically benefit the CNS, were presented by Robertson and colleagues (Abstract 147). Neurocognitive performance was assessed in this randomized placebo-controlled study that compared neuropsychologic testing at baseline, 24 weeks, and 48 weeks among antiretroviral therapy–naive individuals initiating treatment with maraviroc with a placebo or TDF with a placebo plus ritonavir-boosted darunavir and emtricitabine. One hundred nineteen participants were randomly assigned to the maraviroc-containing arm, and 111 were assigned to the TDF-containing arm. Consistent with the findings in the smaller study presented by Winston, there were no differences in global deficit score between the 2 arms at baseline, 24 weeks, or 48 weeks. Both groups improved, but there was no difference in change in score from baseline to 48 weeks between study arms. Overall, 50% of individuals with HAND improved to the unimpaired level with treatment at week 48. These findings may suggest that in the context of the potent immune and viral effects of antiretroviral therapy, any relative benefit of one regimen over another may be too subtle to detect. Studies are ongoing to further examine whether treatment intensification with maraviroc for individuals taking suppressive antiretroviral therapy may cause an improvement in neurocognitive function.

Based on promising data from single-arm studies, Decloedt and colleagues (Abstract 419) performed a randomized placebo-controlled trial of lithium as adjunctive therapy to stable suppressive antiretroviral therapy for treatment of HAND (N = 66). This trial demonstrated improvement over time on repeated neuropsychologic testing but no difference in neurocognitive outcomes between the 2 study arms (those who received lithium and those who received a placebo).

Two studies documented that simplification strategies for antiretroviral treatment appeared safe with regard to neurocognitive outcomes after 1 year of follow-up. Ciccarelli and colleagues (Abstract 417) described 151 participants whose antiretroviral regimen was switched to ritonavir-boosted atazanavir and lamivudine (dual therapy) or who maintained their original 3-drug regimen (triple therapy), as part of the Italian ATLAS-M (Atazanavir and Lamivudine for Treatment Simplification–M) study, and had neuropsychologic testing available. There were no differences in any neuropsychologic testing parameters between the 2 arms at 48 weeks of follow-up. Similarly, Perez-Valero and colleagues (Abstract 424LB) presented data from a neurologic substudy (n = 96) of the SALT study that investigated whether a regimen of ritonavir-boosted atazanavir and lamivudine (dual therapy) was noninferior to a regimen of 2 nucleoside analogue reverse transcriptase inhibitors and ritonavir-boosted atazanavir (triple therapy). At 96 weeks of follow-up, the researchers detected no differences in neurocognitive measures between the 2 groups, although 2 participants in each group developed neurocognitive impairment. These studies suggest that, at least early in follow-up, strategies for antiretroviral treatment simplification may be safe for neurocognitive outcomes in stably treated individuals.

Sacktor and colleagues (Abstract 146) presented results of a double-blind placebo-controlled clinical trial of therapy with paroxetine and fluconazole to address residual inflammation and oxidative stress in the CNS in individuals taking antiretroviral treatment. After screening to identify compounds with potential neuroprotective effects, paroxetine and fluconazole were identified as medications that promoted hippocampal neuron survival in cell culture and protected against neuronal injury in an SHIV model. Individuals with plasma viral suppression were enrolled and underwent neuropsychologic testing at baseline, then were randomly assigned to 1 of 4 treatment arms: fluconazole alone (n = 11), paroxetine alone (n = 11), fluconazole and paroxetine together (n = 12), or a placebo (n = 11). Repeat neuropsychologic evaluation at 24 weeks revealed a benefit in a summarized score of neuropsychologic testing and in a computerized test battery in the paroxetine-containing arms compared with fluconazole alone or a placebo. Depression symptomatology was evaluated at each visit and did not differ between the groups. The group that received fluconazole alone did not exhibit improvement in cognitive testing but did have a greater decrease in CSF ceramide, a measure of oxidative stress, relative to baseline than did the other groups. This is the first adjunctive therapy to demonstrate a beneficial impact on neuropsychologic testing performance in well-treated HIV-infected individuals with HAND. Whether this improvement is attributable to the

One small study demonstrated a benefit of adjunctive therapy with the antidepressant medication paroxetine in individuals taking stable suppressive antiretroviral treatment.

neuroprotective effects of paroxetine or to an undetected impact on mood, the documented benefit of this treatment in the small number of participants studied provides a rationale for a larger clinical trial to examine the potential effect of paroxetine in ameliorating HAND in individuals taking suppressive antiretroviral therapy.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

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Invited Review

CROI 2016: Complications of HIV Infection and Antiretroviral Therapy

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Noncommunicable conditions such as cardiovascular disease, hypertension, renal and bone diseases, and malignancies as well as infectious complications are an ongoing concern during the course of treated HIV disease. Research in this area continues to focus on the epidemiology and risk factors for these conditions, on identifying the contributions of HIV-related immunopathology to specific and collective end-organ diseases, and on evaluating interventions to prevent or reduce the morbidity associated with these conditions. Data presented at the 2016 Conference on Retroviruses and Opportunistic Infections provided new insights into all of these areas.

Keywords: CROI, 2016, complications, HIV, cardiovascular disease, comorbidities, statins, bone, renal, pulmonary, malignancy, tuberculosis, opportunistic infections, cryptococcal meningitis, non–AIDS-related events, atherosclerosis, CVD, CVD risk, adipose tissue, cancer, resistance, screening

New information on the long-term complications of HIV disease remained a major focus at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI), including studies focused on noncommunicable chronic diseases (eg, cardiovascular, renal, and bone diseases) and opportunistic infections (eg, *Mycobacterium tuberculosis* and *Cryptococcus* infections). Progress in identifying predictors of specific complications as well as interventions for their prevention and treatment are reviewed below.

Non–AIDS-Related Events

With the current goal of viral suppression for all people living with HIV infection, new tools are needed to identify those who remain at risk for serious non–AIDS-related events, so that strategies to reduce these complications can be targeted. Previous studies have demonstrated a relationship between lower CD4+/CD8+ cell ratio and the risk for morbidity and mortality caused by non–AIDS-related events.[1] McGettrick and colleagues examined risk factors for non–AIDS-related events among a cohort of individuals taking antiretroviral therapy in Ireland and found that older age at initiation of therapy, injection drug use, and preevent CD4+/CD8+ cell ratio ere independently associated with risk for non–AIDS-related events (Abstract 710). The researchers were able to identify an incremental increase in risk for non–AIDS-related events across a range of CD4+/CD8+ cell ratios; for those with a CD4+/CD8+ cell ratio below 0.26, the hazard ratio (HR) for an event was 3.11 compared with nonsignificant associations for those with ratios above this level. Although options beyond suppressive antiretroviral therapy to raise the CD4+/CD8+ cell ratio are currently limited, these thresholds could be used to target other interventions to reduce non–AIDS-related events in future studies.

Cardiovascular Disease

An analysis of the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) cohort provided more evidence that HIV-infected adults have a higher risk of myocardial infarction (MI) than uninfected controls (Abstract 641). In this analysis, rates of type 1 MI among 7 cohorts of HIV-infected adults in NA-ACCORD were compared with the diverse population of presumably uninfected adults followed in the MESA (Multi-Ethnic Study of Atherosclerosis) and the ARIC (Atherosclerosis Risk in Communities) studies. Adjusted incidence rate ratios for MI in the HIV-infected group ranged between 1.3 (ARIC) and 2.4 (MESA) times higher than in uninfected control groups, after controlling for demographic factors and smoking status but not hypertension or dyslipidemia. These results confirm prior studies that demonstrated an excess risk of MI among adults with HIV infection, using careful control for demographic factors.

The optimal measure for predicting the risk of cardiovascular disease (CVD) remains controversial. This topic was addressed by several well-designed studies (Abstracts 42, 641, 642, and 643). Crane and colleagues used prospectively collected data from the Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort to compare the performance of 4 CVD risk scores: 1) Framingham risk score; 2) Adult Treatment Panel (ATP) III risk score; 3) 2013 American College of Cardiology (ACC)/American Heart Association (AHA) atherosclerotic CVD (ASCVD) risk score; and 4) the HIV-specific CVD risk score from the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study (Abstract 42). The

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investigators also examined the prediction of different types of MI (type 1 due to atherosclerotic plaque and type 2 due to oxygen supply/demand mismatch). In this very large study of 11,338 people with 243 incident MIs, the investigators found that the 2013 ACC/AHA ASCVD risk model performed as well or better than other risk scores across all MI events and that this score outperformed the others for predicting type 2 MI events.

Clement reported on CVD risk prediction using data from the Veterans Affairs Clinical Case Registry that included 3171 male veterans with follow-up over a 10-year period (Abstract 642). In this study, the end points included a broader range of CVD events beyond MI. In contrast to the NA-ACCORD study, the D:A:D model performed better than the ACC/AHA ASCVD risk calculator, and inclusion of hepatitis C virus infection and HIV RNA into a new model improved discrimination.

Weigel compared the 2013 ACC/AHA guidelines[2] on ASCVD risk with the 2004 ATP III recommendations[3] in 352 individuals without prior ASCVD who had available data on the presence of carotid plaque and 3-year rates of progression of carotid intima-media thickness (CIMT), to determine whether the guidelines recommended the use of statins in those with evidence of disease. The investigators found that although the 2013 ACC/AHA guidelines recommended statins to a greater percentage of individuals with carotid plaque, the 2004 guidelines better correlated with individuals with progression of CIMT. Whether the inclusion of a measure of subclinical atherosclerosis such as CIMT would improve longer-term risk prediction remains to be determined. Further improvements in HIV risk prediction can likely be obtained with fine tuning of the available risk equations. In the interim, the use of the 2013 ACC/AHA ASCVD risk score for predicting MI is unlikely to overestimate CVD risk.

The role of noninvasive cardiovascular testing to stratify coronary artery disease risk among individuals with HIV infection also remains poorly defined. Feinstein and colleagues (Abstract 644) used a clever approach to obtain data on this question by reviewing the outcome of stress test results within the Northwestern University database. Among individuals with a positive stress test result, those with HIV infection were more likely to have a greater burden of coronary artery disease during angiography and a higher rate of percutaneous coronary interventions. Whether this represents a difference in testing rates or a higher rate of subclinical disease awaits further study.

Further improvements in HIV risk prediction can likely be obtained with fine tuning of the available risk equations. In the interim, the use of the 2013 ACC/AHA ASCVD risk score for predicting MI is unlikely to overestimate CVD risk.

Biomarkers of Atherosclerosis and CVD Risk

Circulating biomarkers that predict the risk of MI or other measures of subclinical atherosclerosis help to reveal the pathogenesis of these problems and to identify potential targets for interventions as well as individuals at high risk. Numerous studies examined the relationship between a variety of plasma biomarkers and cellular markers and a range of different cardiovascular outcomes, including measures of endothelial function, CIMT, and clinical events (Abstracts 671-673 and 651-657). It is challenging to summarize and reconcile the findings from these studies, given the lack of standardized practices for controlling for other risk factors within each study and the fact that each type of end point could reflect a different mechanistic process. Despite this limitation, these findings highlight important insights into the pathogenesis of these clinical problems.

Hunt and colleagues found that along with C-reactive protein and D-dimer, higher levels of oxidized low-density lipoprotein (LDL) cholesterol were associated with MI (Abstract 671). Nou and colleagues demonstrated that the reduction in coronary artery plaque (as measured by coronary computed tomography angiography) observed during a clinical trial of atorvastatin therapy paralleled a reduction in oxidized LDL cholesterol, suggesting that the effect of the statin was mediated through this mechanism (Abstract 673). Collectively, these studies highlight the potential role of oxidized lipids in the pathogenesis of atherosclerosis in the context of HIV infection. In contrast, Kelesidis and colleagues did not find a statistically significant association between oxidized LDL and progression of CIMT in individuals who initiated antiretroviral therapy over 96 weeks (Abstract 672), possibly suggesting that the effects of oxidized lipids may not be evident in the CIMT measurement. Other factors that were associated with the risk of clinical MI events included higher levels of B-type natriuretic peptide (Abstract 646).

Immune Activation: Unique Roles for T Cells and Monocytes

Specific monocyte subpopulations (CD14+ + CD16+) and expression of surface markers have been associated with levels of plasma biomarkers of inflammation and with a higher risk for subclinical atherosclerosis.[4,5] Chow and colleagues (Abstract 652) reported an association between carotid bifurcation thickness and both the nonclassical monocyte population (CD14low/ + CD16+ +) and monocyte chemotactic protein–1 (MCP-1) and 2-year rates of coronary artery calcification in the Hawaii Aging With HIV study (Abstract 652). These results confirm the growing literature on the importance of innate immune activation and progression of atherosclerosis in HIV infection.

Crowe and colleagues previously showed that glucose metabolism within monocytes remains altered after antiretroviral therapy.[6] At CROI 2016, Palchaudhuri and colleagues demonstrated that higher expression of glucose transporter 1 (GLUT1) on intermediate proinflammatory monocytes (CD14+ + CD16+) was associated with higher levels of some plasma biomarkers of CVD risk (D-dimer and lower levels of high-density lipoprotein [HDL] cholesterol), suggesting that inhibitors of GLUT1 could be targeted to reduce inflammation mediated in treated HIV infection (Abstract 711).

Several studies examined the role of activated T cells among individuals with MI and in relation to measures of endothelial function and markers of endothelial activation (Abstracts 653, 654, and 655). Higher levels of CD8+ T cells expressing CC chemokine receptor 5 were observed prior to acute coronary syndrome, suggesting a possible therapeutic target (Abstract 655). When endothelial dysfunction was examined as the end point (as measured by flow-mediated dilation of the brachial artery), one small study reported an association between activated CD8+ T cells and flow-mediated dilation (Abstract 653), and another study noted stronger links between cytomegalovirus immunoglobulin G and cytomegalovirus-specific CD4+ T cells (Abstract 654). Measures of monocyte activation appeared to be more closely related to endothelial activation markers (intercellular adhesion molecule–1 [ICAM-1] and vascular cell adhesion molecule–1 [VCAM-1]), and T-cell activation was not related to this outcome measure. Taken together, these findings suggest that endothelial activation and vascular reactivity are influenced through different mechanisms. For a summary of the complex topic of immunopathogenesis of metabolic complications, see the webcast of the symposium talk by Crowe (Abstract 125).

Vascular Function and Antiretroviral Therapy

The degree to which early antiretroviral therapy reduces CVD risk remains unknown. Baker and colleagues compared vascular function between individuals with untreated HIV infection randomly assigned to early antiretroviral therapy (initiated at CD4+ cell counts >500/μL) in the START (Strategic Timing of Antiretroviral Treatment) study, by measuring radial artery waveforms with a tonometer (Abstract 41). The 332 participants in this substudy were young, predominantly men, and had low CVD risk at baseline. During a median follow-up period that included 30 months of antiretroviral therapy for the immediate-treatment group, there were no within-person or between-group differences in vascular function. These results suggest that among a population of young, healthy people, early antiretroviral therapy was not able to impact this specific measure of vascular function.

In contrast, Innes and colleagues examined a different measure of vascular function, aortofemoral pulse wave velocity (PWV; a measure of elevated arterial wall stiffness), in children who initiated antiretroviral therapy early in life compared with a demographically similar HIV-uninfected group (Abstract 658). The investigators demonstrated that PWV improved consistently among the HIV-seropositive group with a longer duration of antiretroviral therapy, whereas there were no changes in PWV with age in the control group. These results suggest that vascular function may improve over time with early initiation of antiretroviral therapy.

Interventions to Reduce Inflammation and CVD Risk

O'Brien and colleagues from the AIDS Clinical Trials Group (ACTG) performed a randomized controlled trial to follow up on an earlier uncontrolled observation that low doses of aspirin might reduce levels of innate immune activation in the setting of well-treated HIV infection (Abstract 44LB). In this trial, 100 mg and 300 mg doses of aspirin were compared with placebo during 12 weeks of treatment followed by a washout period. Although serum thromboxane was inhibited among those who received aspirin (suggesting participants were adherent), there was no impact of the aspirin intervention on soluble CD14 or on flow-mediated dilation of the brachial artery. In fact, one measure of monocyte activation (soluble CD163) rose in the group that received aspirin. The study population was not preselected for a group with elevated measures of immune activation at baseline. Hence, it is still possible that there may be subgroups for whom aspirin therapy may reduce inflammation, and this can hopefully be explored in future studies.

Cardiovascular Disease Among Women

CVD risk among women was the focus of a few studies at CROI 2016. Hanna and colleagues from the Women's Interagency HIV Study compared the use of interventions to reduce CVD risk among HIV-seropositive with that among HIV-seronegative women and found that HIV-seropositive women with hypertension and diabetes were more likely to be on treatment for these issues than were HIV-seronegative controls; however, a sizable fraction did not achieve target levels of control for either condition (Abstract 647).

Looby and colleagues used stored samples from a study that measured the burden of coronary artery plaque by computed tomography scan and examined the relationship between menopausal status, plaque burden, and measures of innate immune activation (Abstract 650). Using a novel marker of ovarian reserve, anti-Müllerian hormone (a measure of reproductive aging), they were able to show that lower levels of anti-Müllerian hormone were associated with higher plaque burden and with higher levels of innate immune activation. These results suggest a possible connection between reproductive aging and CVD risk in the context of HIV infection. These findings also highlight the need to prioritize CVD screening among HIV-infected women prior to the onset of clinical menopause.

Heart Failure

There is growing awareness of heart failure as an important clinical problem in the setting of treated HIV infection, including among children (Abstract 853). Factors that contribute to a decline in left ventricular function during HIV infection are poorly defined. Longenecker reported an association between the presence and quality of pericardial fat depots and left ventricular function in a study of 46 individuals that combined echocardiographic measures with findings on computed tomography scanning (Abstract 649). This novel finding of ectopic fat depots and diastolic function warrants further evaluation. Previous studies have demonstrated a link between depression and heart failure.[7] In a comprehensive study of US veterans, this link was confirmed for HIV-infected and uninfected men (Abstract 714).

Bone Disease

It is well established that bone mineral density (BMD) initially declines after the initiation of antiretroviral therapy, but the impact of this change on long-term risk for fracture and the contribution of specific antiretroviral drugs to fracture risk remain unclear. Borges and colleagues examined rates of fracture and femoral osteonecrosis among nearly 12,000 participants from the EuroSIDA study (Abstract 46). The investigators identified that well-known host factors such as age, race, and HIV disease status were associated with fracture risk; however, after controlling for these factors, use of tenofovir disoproxil fumarate (TDF) (but not duration of exposure) was an independent risk factor for fracture. The excess risk of fracture ranged from 25% for those who had ever received TDF to 40% for those currently taking TDF. This is one of the largest studies to date to quantify the relationship between TDF exposure and fracture risk.

Interventions to reduce fracture risk have included changing the antiretroviral regimen and the use of pharmacologic agents to restore bone density. Gallant and colleagues presented data from a clinical trial that randomly assigned individuals who were virologically suppressed on a regimen that contained emtricitabine and TDF to remain on the same regimen or to switch the TDF component to tenofovir alafenamide (TAF) (Abstract 29). After 48 weeks of follow-up, those who switched to the TAF-containing regimen remained virologically suppressed, and BMD increased in the group receiving TAF but declined in the group receiving TDF. Additionally, more participants in the group receiving TAF had an improvement of 3% or more in BMD at week 48. These results confirm that TAF may have more favorable effects than TDF on bone. Further study is needed to determine the clinical significance of these changes.

Ofotokun and colleagues examined the impact of a single dose of zoledronic acid, an injectable agent that inhibits osteoclast activity and reduces bone turnover, to prevent bone loss among individuals initiating antiretroviral therapy (Abstract 47). Antiretroviral therapy–naive participants who were beginning a regimen of ritonavir-boosted atazanavir plus TDF and emtricitabine were randomly assigned to receive a single dose of zoledronic acid 5 mg or a placebo and followed for 48 weeks. After 12 weeks of follow-up, bone loss (as measured by C-terminal telopeptide of collagen [CTx], a sensitive marker of bone resorption) was reduced by 74% in the group receiving zoledronic acid. The benefits of zoledronic acid were maintained over 48 weeks of follow-up, and the drug was well tolerated. If confirmed in larger studies, perhaps among patients receiving contemporary antiretroviral regimens, these findings highlight a promising approach to preventing bone loss when initiating antiretroviral therapy.

Injectable zoledronic acid reduced bone loss by 74% among individuals taking an antiretroviral regimen of ritonavir-boosted atazanavir plus TDF and emtricitabine.

There is also a need to identify the most effective strategies to prevent bone loss among youth who are being treated for HIV infection. In a randomized clinical trial that evaluated different dosage strategies for vitamin D treatment, Eckard and colleagues found that a monthly dose of vitamin D_3 60,000 IU was effective in improving BMD and reducing levels of markers of bone turnover in individuals aged 8 years to 26 years taking antiretroviral therapy with a baseline 25-hydroxyvitamin D level of 30 ng/mL or lower (Abstract 859).

Questions remain about the safety of maternal use of TDF and newborn BMD. Siberry and colleagues from the International Maternal, Pediatric, Adolescent AIDS Clinical Trials (IMPAACT) Network PROMISE (Promoting Maternal-Infant Survival Everywhere) study compared bone outcomes among infants born to pregnant women enrolled in a 3-arm randomized trial (Abstract 36). The trial compared an antiretroviral regimen of ritonavir-boosted lopinavir and TDF or zidovudine with maternal prevention of mother-to-child transmission (PMTCT) prophylaxis with zidovudine, single-dose nevirapine, and a "tail" of TDF and emtricitabine. Using whole-body and lumbar spine dual-energy X-ray absorptiometry (DXA) scanning, the investigators measured bone mineral content (BMC) by age 28 days at 8 African sites. Among the 425 infants with DXA data available, the investigators found no difference in BMC among those whose mothers were taking boosted lopinavir and TDF or zidovudine; however, levels of BMC were higher in infants exposed to the short-course PMTCT regimen than in those exposed to maternal 3-drug antiretroviral therapy. The clinical significance of this difference over the longer term remains to be identified. Overall, these results provide reassurance that maternal exposure to TDF does not lead to lower BMC than exposure to zidovudine, at least when combined with boosted lopinavir.

Further evidence that ritonavir-boosted lopinavir may play a role in bone loss was seen in a study of children in South Africa who were receiving boosted lopinavir and were randomly assigned to continue or to switch to an efavirenz-based regimen (Abstract 40). After an average of 2 years of follow-up, levels of BMC were lower in the children who remained on boosted lopinavir than in those who switched to efavirenz, even after controlling for several factors associated with BMC.

The use of oral preexposure prophylaxis (PrEP) containing emtricitabine and TDF has been associated with a reduction in BMD in men.[8] This is of particular concern for healthy young men who have not yet achieved peak bone mass. Grant and colleagues presented follow-up data from a bone density substudy of the iPrEx (Chemophrophylaxis for HIV Prevention in Men) study and the companion follow-up iPrEx OLE (iPrEx Open Label Extension) study (Abstract 48LB). In this report, the investigators examined changes in BMD among those participants with measureable levels of emtricitabine and TDF 24 weeks after treatment was discontinued and observed reversal of bone loss back to levels comparable to those in the placebo group over this period of time. The findings from this study confirm that bone loss

occurs during PrEP with emtricitabine and TDF (with drug levels needed for protection from HIV-1 infection) and that this loss reverses 6 months after treatment is discontinued. For a comprehensive review of the topic of bone disease and HIV infection, see the webcast of the symposium talk by Mallon (Abstract 126).

Renal Disease

A very large study from NA-ACCORD investigators examined changes in estimated glomerular filtration rate (eGFR) among individuals taking TDF compared with other nucleoside analogue reverse transcriptase inhibitors (nRTIs) and found that, among those with a baseline eGFR below 90 mL/min, renal function declined more quickly over the first 6 months of therapy with TDF than with other nRTIs but then recovered only in those taking TDF (Abstract 684). In contrast, no differences in renal function were observed between those using TDF and those using other nRTIs if the baseline eGFR was greater than 90 mL/min. These results confirm transient declines in renal function during initial antiretroviral therapy containing TDF among those with mild renal impairment.

TAF, a prodrug of tenofovir, achieves lower plasma levels of tenofovir than TDF with equivalent or higher levels of intracellular tenofovir diphosphate and, hence, is expected to cause fewer renal and bone adverse effects. Several studies evaluated the safety of TAF among individuals at risk for renal disease or with established renal impairment. Wohl and colleagues reported the results of 2 prospective studies that compared TAF-containing with TDF-containing initial antiretroviral regimens and examined changes in renal function in those with 2 or more risk factors for renal disease and in those without risk factors (Abstract 681). In individuals at risk for renal disease, a decline in renal function was less common among those who received TAF than those who received TDF.

In individuals at risk for renal disease, a decline in renal function was less common among those who received TAF than those who received TDF.

Safety data on the use of TAF through 72 weeks in patients with impaired renal function (eGFR 30-69 mL/min) were presented by Post and colleagues (Abstract 680). In this study, individuals with renal impairment were switched to the fixed-dose combination of elvitegravir, cobicistat, TAF, and emtricitabine. In addition to continued stabilization of eGFR, improvement in BMD and reductions in proteinuria and albuminuria were also observed. These data provide reassurance about the use of TAF to reduce the risk of developing renal dysfunction among individuals at high risk for renal disease as well as among those with preexisting renal impairment.

Adipose Tissue

Damouche and colleagues expanded on prior observations[9] describing a possible role of visceral fat as an anatomic location for HIV persistence and as a source of chronic inflammation (Abstract 271). In elegant studies using adult cynomolgus macaques (*Macaca fascicularis*) infected with simian immunodeficiency virus $(SIV)_{mac251}$ and uninfected animals as controls, the investigators provided evidence that lymphocytes and macrophages isolated from adipose tissue and the stromal vascular fraction of this tissue harbored SIV DNA and RNA even in the presence of suppressive antiretroviral therapy. These findings suggest that adipose tissue may play a role in both viral persistence and chronic immune activation and inflammation during HIV infection.

New information continues to emerge regarding the contributions of specific antiretroviral drugs to changes in fat. Data from a small randomized trial that included samples from subcutaneous fat biopsies demonstrated a greater reduction in mitochondrial DNA in adipose tissue among individuals who were randomly assigned to receive zidovudine combined with a nonnucleoside analogue reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (Abstract 668). These results highlight the possible contributions of NNRTIs to fat loss.

Investigators from the European AIDS Treatment Network (NEAT) presented the results of a body composition substudy nested into a study of treatment with ritonavir-boosted darunavir combined with raltegravir (nRTI-sparing regimen) or TDF and emtricitabine (Abstract 45). No changes in limb fat were observed between treatment groups, but total body fat mass and mean trunk mass increased more in those who were taking the nRTI-sparing regimen. Whether this increase in fat mass resulted from the lack of nRTIs or from the use of raltegravir cannot be determined from this design. For a summary of the topic of visceral fat in the context of HIV infection, see the webcast of the symposium talk by McComsey (Abstract 123).

In summary, considerable progress is being made in deciphering the unique factors that contribute to the risk of several long-term complications of chronic diseases in the setting of treated HIV infection. Although it appears that earlier initiation of antiretroviral therapy may not have a major impact on the risk of some of these issues, more attention to screening and prevention of comorbidities along with improvements in antiretroviral therapy and the development of specific interventions to reduce immune activation will be needed to eliminate these conditions as a source of comorbidity globally.

Cancer

People living with HIV infection are at increased risk for human papillomavirus (HPV)-related cancer. Wilkin and colleagues presented data from the ACTG A5298 trial, a randomized, double-blind, placebo-controlled trial of the quadrivalent HPV vaccine among HIV-infected adults aged 27 years or older (Abstract 161). Five hundred seventy-five adults were enrolled in the trial, with a median age of 47 years; participants were followed up for a median of 2.6 years. A data and safety monitoring board stopped the study early for futility. The vaccine did not prevent persistent anal HPV

infections compared with placebo (13 vs 17, respectively; HR, 0.75; 95% confidence interval [CI], 0.45-1.26). In addition, the vaccine did not have an impact on the occurrence of anal high-grade squamous intraepithelial lesions (HSILs), the precursor to invasive anal cancer. Vaccinated participants experienced fewer persistent oral HPV infections than those who received a placebo (1 vs 8, respectively; HR, 0.22; 95% CI, 0.02-0.98). The vaccine was safe and immunogenic, consistent with earlier studies. The investigators suggested that the vaccine failed to prevent anal HPV infection because the population had been highly exposed to HPV, and the new infections may have been present at very low levels at baseline. The prevention of oral HPV infections is an important finding, as there are currently no accepted strategies for prevention of HPV-related oropharyngeal cancer.

Borges and colleagues presented data on the occurrence of cancer in the START study (Abstract 160). In the START trial, antiretroviral therapy–naive participants with CD4+ cell counts above 500/µL were randomly assigned to immediate initiation of antiretroviral therapy or delay of therapy until their CD4+ cell count declined to 350/µL or until there was another indication for antiretroviral therapy. Among participants, the overall rate of cancer decreased by 64% (14 vs 39 cancers, respectively; HR, 0.36; 95% CI, 0.19-0.66; P = .001), and risk reduction was greatest for infection-related cancer (6 vs 23, respectively; HR, 0.26; 95% CI, 0.11-0.64; P = .003) (cancers were predominantly Kaposi sarcoma and lymphoma). There was a nonsignificant decrease in cancer unrelated to HIV infection (8 vs 16, respectively; HR, 0.49; 95% CI, 0.21-1.15; P = .1). The effect of early antiretroviral therapy on HIV infection–related cancer did not appear to be mediated by suppression of plasma HIV-1 RNA, whereas viral suppression did mediate the effect of antiretroviral therapy on cancer unrelated to HIV infection. The investigators suggested that other factors such as reduced inflammation or a direct effect of antiretroviral therapy on viral coinfections were responsible for the reduced risk of cancer related to HIV infection.

Recent guidelines for the prevention of cervical cancer have advocated less frequent screening.[10] Silverberg and colleagues conducted a retrospective review of cervical HSILs or cancer in a large health system database (Abstract 162). The investigators conducted a large case-control study to examine the risk factors for these events, including HIV infection. HIV-infected women were 2.3 times more likely to have cervical HSILs or cancer than uninfected women. However, HIV-infected women with CD4+ cell counts above 500/µL had similar risk to that of uninfected women. This suggests that HIV-infected women with high CD4+ cell counts may not require more intensive cervical cancer screening.

Opportunistic Infections

Strategies to Reduce Tuberculosis-Associated Mortality Among Persons With HIV Infection

Tuberculosis (TB) remains the leading killer of persons living with HIV infection worldwide. Delays in TB diagnosis and treatment contribute to this mortality. Two randomized studies sought to show that new TB diagnosis and treatment strategies could reduce this mortality. Novel strategies in both studies failed to reduce mortality. Notably, both studies included timely initiation of antiretroviral therapy. The results were disappointing, somewhat surprising, and highlight the need for better strategies for individuals who present to care with low CD4+ cell counts and the priority of initiating antiretroviral therapy earlier in the course of HIV disease, when TB is much less frequent and less fatal.

The TB Fast Track study randomly assigned 24 clinics in South Africa to 1 of 2 arms: a treatment using a "fast-track" TB clinical algorithm or the standard of care (Abstract 155). Adults with CD4+ cell counts below 150/µL were eligible for participation. In the fast-track arm, participants were categorized as having high, medium, or low risk for TB based on clinical characteristics associated with TB (reduced hemoglobin level and body mass index) and results of a rapid urine lipoarabinomannan (LAM) test. Participants at high risk for TB received immediate TB treatment followed by antiretroviral therapy after 2 weeks. Participants with medium risk had further diagnostic tests, and participants with low risk initiated antiretroviral therapy. Of participants, 45.7% were classified as high risk, 31.5% as medium risk, and 22.8% as low risk. Mortality at 6 months, for the 3030 participants enrolled in the study, did not differ between the 2 arms: 19.0 per 100 person-years (fast track) and 21.5 per 100 person-years (standard of care) (adjusted risk ratio, 0.87; 95% CI, 0.61, 1.24). Antiretroviral therapy was delayed in the intervention arm despite the intent of the study to accelerate the life-saving interventions of TB and HIV treatments.

A "fast-track" TB treatment algorithm for high-risk individuals failed to reduce mortality in a South African study.

The REMEMBER (Reducing Early Mortality & Morbidity by Empiric TB Treatment) study randomly assigned participants screened for TB based on clinical symptoms and rapid sputum tests to either empiric TB treatment or isoniazid preventive therapy (Abstract 745). The rationale for this study was that current clinical and microbiologic rapid diagnostic assessments are too insensitive to identify individuals with low CD4+ cell counts at the highest risk for TB mortality, and that empiric 4-drug TB therapy could reduce this mortality. Eight hundred fifty participants predominantly from Africa were enrolled, with a median CD4+ cell count of 18/µL. At 1 year, mortality was 7.2% with empiric TB treatment and 8.7% with isoniazid preventive therapy, and the probability of AIDS or death was 19.3% and 15.3%, respectively.

Empiric TB treatment in individuals with low CD4+ cell counts in low-income settings failed to reduce rates of mortality.

TB was significantly more frequent among individuals who received 4-drug TB therapy than those who received isoniazid preventive therapy (5.6% vs 2.4%, respectively; $P = .02$). The investigators postulated poorer adherence to 4-drug TB therapy than to isoniazid preventive therapy as one possible explanation for these results.

To address the possibility that screening of participants by urine LAM test could have affected the study results, Bisson evaluated baseline stored urine LAM samples from 67% of participants in the REMEMBER study (Abstract 747). Urine LAM testing would have identified and excluded 28 additional participants in the study. However, after these participants were excluded in a secondary analysis, the same trends reported in the primary analysis were found.

In a related study on TB mortality, Manabe examined mortality outcomes among 804 HIV-infected adults with a presumptive diagnosis of TB enrolled in a TB diagnostic study that used extensive microbiologic tests for final classification (Abstract 743). Of participants, 78% were hospitalized, and 43% were ultimately classified as having TB infection. Six-month mortality rates were high in those with TB and those without TB (26.4% and 26.6%, respectively). Median CD4+ cell count was higher among those without than with TB (114/μL for those without TB vs 59/μL for those with TB). These data show that individuals with TB-like syndromes but without TB require new strategies to reduce mortality rates.

TB among children is even more challenging to diagnose and treat. In an analysis of the International Epidemiologic Databases to Evaluate AIDS (IeDEA) cohort, Carlucci reported that among 295 children (median age, 5.7 years; interquartile range, 2.0-9.6) treated for TB, 22% had unfavorable outcomes (ie, death, treatment failure, or loss to follow up), and outcomes were similarly poor to those of children with and without confirmed microbiologic TB diagnoses (21%).

Extensively Drug-Resistant TB, Multidrug-Resistant TB, and Meropenem for TB Treatment

Understanding of transmission of extensively drug-resistant (XDR) TB is needed to design strategies to halt its spread. Auld described a social network analysis of XDR TB in KwaZulu-Natal, South Africa, from 2010 to 2014 (Abstract 157). The researchers defined a social network connection or an overlapping hospitalization as an epidemiologic link. Among 404 participants with XDR TB, 58% were women, 77% were infected with HIV, and the median age was 34 years. Epidemiologic links were identified in 287 (71%) participants. Of these, 92% were in the same home and 66% had overlapping hospitalizations. These data indicate that the XDR TB epidemic is being driven by transmissions in households and hospitals. Focusing contact tracing at households and limiting exposure of individuals with XDR TB to uninfected persons in hospitals are strategies supported by these findings.

In a South African study, XDR TB was determined to be driven by transmission in households.

Treatment of XDR and multidrug-resistant (MDR) TB often includes injectable aminoglycosides, which are associated with high rates of adverse events, including ototoxic effects. Individuals with HIV infection also require antiretroviral therapy, which may include TDF, a drug that can cause nephrotoxic effects. Brust reported on toxic effect outcomes among 206 individuals with XDR TB (Abstract 756). Among these individuals, 150 had HIV infection and all were receiving antiretroviral therapy. More than half (56%) of individuals experienced hearing loss (≥ grade 1), and 9% experienced severe hearing loss (≥ grade 3). Forty percent developed hypothyroidism requiring replacement therapy. Electrolyte imbalances were common; renal failure was not reported. Persons with HIV infection taking antiretroviral therapy did not experience more toxic effects than persons without HIV infection. These findings highlight that current XDR TB regimens are associated with clinically significant rates of toxic effects but that individuals with HIV infection who are treated with antiretroviral therapy do not experience worse toxic effects than their uninfected counterparts.

Shin and colleagues evaluated rates of treatment success among 403 adults with MDR TB according to HIV infection and antiretroviral therapy status in Botswana from 2006 to 2013 (Abstract 755). Treatment success (microbiologic cure) was similar between HIV-uninfected (82.3%) and HIV-infected (81.0%) individuals taking antiretroviral therapy. Success rates were only 55% among those with HIV infection who were not taking antiretroviral therapy. Mortality rates were lower among HIV-infected individuals treated with antiretroviral therapy than those not treated, but treatment failure was still unacceptably high in all groups (27.5% among HIV-infected persons not taking antiretroviral therapy, 19.2% among HIV-infected persons taking antiretroviral therapy, and 13.2% among uninfected persons).

The high rates of mortality, treatment failure, and toxic effects associated with treatment of XDR and MDR TB in HIV-infected and uninfected persons call attention to the urgent need for new TB treatments. Diacon presented the results of a 2-week intensive microbiologic study examining meropenem and faropenem (Abstract 158LB). The trial tested the hypothesis that meropenem 2 g given intravenously every 8 hours or faropenem 600 mg given orally every 8 hours, each in conjunction with a β-lactamase inhibitor (clavulanic acid), could achieve sufficient plasma levels to inhibit *M. tuberculosis* replication. Because clavulanic acid was not available as a sole agent, it was provided in a coformulated preparation with amoxicillin. In the control (standard TB therapy) and meropenem groups, viable microbiologic load in sputum at 2 weeks was lowered by 0.17 $\log_{10}$

Meropenem plus amoxicillin coformulated with clavulanic acid showed microbiologic activity against TB in an intensive 2-week study.

colony-forming units (CFU)/mL and 0.11 $\log_{10}$ CFU/mL, respectively. There was no reduction observed in the individuals in the faropenem arm, presumably because of the low levels of faropenem achieved with the oral preparation. Most toxic effects observed were gastrointestinal symptoms, attributed by the investigators to amoxicillin and clavulanic acid. These data show proof of principle that meropenem given at this dose with amoxicillin and clavulanic acid can reduce TB levels in 2 weeks, in the range of other potent TB regimens. Identifying carbapenems with activity against TB that can achieve therapeutic levels with oral dosing should be a high priority in the development of TB treatments.

Population-Level Screening for TB

TB symptoms are often present months before an individual presents to a health facility. In the context of the large ongoing PopART (Population Effects of Antiretroviral Therapy to Reduce HIV Transmission) study, an HIV test-and-treat study being conducted in South Africa and Zambia, investigators reported on the uptake and yield of adding TB screening to household HIV testing (Abstract 156). Study staff obtained consent from household members for TB screening at the time of household HIV testing. Sputum was collected for microbiologic diagnosis among persons with TB symptoms. The investigators performed TB screening for 209,429 of 212,819 consenting household members. Of these, 2538 reported TB symptoms, and 1918 (79.3%) had TB test results; 167 of 1918 (8.7%) had a positive TB test result. The investigators concluded that home screening identified TB in nearly 9% of the population and was effective in reaching these individuals before they presented to a health center.

In a household-based TB screening study conducted in 8 communities (103,000 persons) in Haiti, many undiagnosed cases of TB were identified (Abstract 739). Community health workers identified 6926 individuals with suspected TB (7% of the population). Chronic cough was confirmed at a physician visit in 3397 (49%) individuals. Of the 3147 (93%) persons who received HIV and TB microbiologic tests, 302 were HIV infected; 90 (30%) of the 302 HIV-infected persons were diagnosed with TB. Of HIV-uninfected persons, 22% were found to have TB. In this area with high HIV and TB prevalence, household screening and testing were highly effective in identifying undiagnosed cases of these infections.

In Haiti, household screening and testing were highly effective in identifying undiagnosed cases of HIV and TB infections.

In a second analysis from Haiti, Rivera and colleagues evaluated the addition of clinical and microbiologic screening (acid-fast bacilli smear, Xpert MTB/RIF assay, and TB culture) to a routine HIV testing program (Abstract 750). Among 30,316 persons presenting for HIV testing, 3252 (11%) were HIV infected. Among these, 1081 (33%) reported a cough. Among those who reported a cough, 245 (23%) were diagnosed with TB. Sixty-seven percent of the TB diagnoses were confirmed microbiologically. Testing with the Xpert MTB/RIF assay, a rapid combined TB and resistance to rifampicin assay, increased the bacteriologically confirmed diagnoses of TB by 30%. These data illustrate the opportunity for rapid TB diagnosis among persons presenting for HIV testing and the increased yield that may be achieved by incorporating testing with the Xpert MTB/RIF assay.

Cryptococcal Disease

Identifying and treating cryptococcal disease in severely immunosuppressed HIV-infected individuals before they develop clinical meningitis, in populations with a high prevalence of cryptococcal antigenemia, is now recommended as one approach by the World Health Organization.[11] Longly described the outcome of practitioner-initiated screening for cryptococcal antigen (CrAg) among individuals with CD4+ cell counts below 100/µL in Cape Town, South Africa, from 2012 to 2013 (Abstract 759). The protocol called for treatment with oral fluconazole for asymptomatic individuals who tested positive for CrAg. Only 1170 (26.6%) of 4395 eligible individuals were screened for CrAg. The prevalence of cryptococcal antigenemia among screened individuals was 2.1% (24/1170). Information on use of fluconazole was available for 13 of the 24 individuals with cryptococcal antigenemia. Among these, 9 of 13 received fluconazole and none developed cryptococcal meningitis. During the observation period, there were 9 cases of disseminated cryptococcal disease, many of which in individuals who had delays in initiation of antiretroviral therapy. Antiretroviral therapy was initiated for 72% of persons screened and only 48% of persons not screened for CrAg. The investigators appropriately concluded that an implementation approach that relied on practitioner-initiated screening for CrAg without training or feedback was unsuccessful. These results also illustrate that delays in initiation of antiretroviral therapy among persons with low CD4+ cell counts remain a persistent issue.

In a related abstract, Letang reported a more successful approach to CrAg screening (Abstract 760). At the St. Francis referral hospital in Tanzania, "reflex" CrAg screening was performed on all inpatients and outpatients with CD4+ cell counts below 150/µL from 2013 to 2015. Five hundred patients were screened. The prevalence of CrAg was 12% among inpatients and 5.3% among outpatients. Among the cases in which CrAg was identified, a lumbar puncture was performed within 1 day for 31 of 32 individuals; 39% (12/31) had microbiologic evidence of cryptococcal meningitis. Interestingly, 2 of the 12 individuals with evidence of cryptococcal meningitis had no reported neurologic symptoms. The mortality rate of those with cryptococcal meningitis was 86% over the study time period. Mortality among other individuals who tested positive for CrAg (81% were treated with fluconazole) was similar to that among those who tested negative for CrAg (9%).

Predictors of progression from the time of CrAg detection to onset of cryptococcal meningitis are poorly understood.

In the context of an ongoing study on the use of fluconazole for individuals with CrAg, Morawski presented data on outcomes for individuals with detectable CrAg (Abstract 159). Individuals with CrAg and no signs or symptoms of meningitis were treated with fluconazole 800 mg for 2 weeks, followed by fluconazole 400 mg for 8 weeks. In this study population of 151 persons, the median CrAg titer was 1:40. Death occurred in 13.9% of the cohort; 23.2% of participants met the primary end point of death or cryptococcal meningitis. The CrAg titer was highly associated with progression to the primary end point, with a 2.5 greater risk for meningitis when the CrAg titer was greater than 1:160. A CD4+ cell count below 50/μL was the most powerful predictor of outcome. These data advance the understanding of cryptococcal disease progression in the face of a punctuated treatment with fluconazole. These data also raise many additional questions regarding the optimal clinical approach to treatment of individuals with CrAg, including the treatment regimen, treatment duration, and the optimal criteria to tailor treatment for those at the highest risk.

Whether the presence of CrAg without meningitis is associated with neurocognitive changes is unknown. Montgomery and colleagues evaluated neurocognitive function among individuals in African cohorts in 3 groups: 1) those with cryptococcal meningitis (n = 90); 2) those in whom cryptococcal antigenemia was detected (n = 87), with no meningitis; and 3) those with no cryptococcal disease (n = 125) (Abstract 761). The researchers performed a standardized neurocognitive function evaluation at the time of the initiation of antiretroviral therapy and then 4 weeks after the initiation of treatment with fluconazole in group 2. Median CD4+ cell counts and Karnofsky scores among groups 1, 2, and 3, respectively, were 17/μL and 60, 26/μL and 70, and 223/μL and 90. Composite neurocognitive function was lowest among individuals with cryptococcal meningitis, and it was lower among individuals with CrAg only than among HIV-infected individuals with no cryptococcal disease; however, CD4+ cell counts were different among these groups. Neurocognitive function in the cohort of 87 persons with CrAg but without meningitis improved to within 1 standard deviation of persons without cryptococcal disease, 4 weeks after treatment with fluconazole and antiretroviral therapy. It is difficult to assess whether this improvement was attributable to fluconazole, antiretroviral therapy, or both.

Boulware and colleagues conducted an analysis of individuals with cryptococcal disease to determine if clinical and immune parameters could help distinguish immune reconstitution inflammatory syndrome (IRIS) from disease relapse (Abstract 762). The investigators applied a standard definition of IRIS and used cultures from cerebrospinal fluid to define relapse. Among the cohort analyzed, 70 persons had 75 episodes of recurrent meningitis, 62 of which were classified as IRIS and 13 of which were classified as disease relapse. None of clinical lab parameters (CD4+ cell count, HIV RNA level) examined distinguished between these 2 clinical scenarios, which call for different treatment approaches. Interleukin (IL)-13 (a cytokine associated with uncontrolled cryptococcal disease in murine models) in cerebrospinal fluid was 35-fold higher in those with disease relapse than in those with IRIS. Other inflammatory markers (interferon-γ, IL-4, and IL-17) were higher in those with IRIS than those with disease relapse. Without fungal cultures, it remains difficult to distinguish between IRIS and relapse of cryptococcal disease. The investigators noted that the best way to reduce disease relapse is optimal treatment of cryptococcal meningitis. Many places in Africa still treat cryptococcal meningitis with fluconazole alone because of a lack of access to amphotericin B.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

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Invited Review

CROI 2016: Viral Hepatitis and Liver Fibrosis

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At the 2016 Conference on Retroviruses and Opportunistic Infections (CROI) in Boston, Massachusetts, hepatitis C virus (HCV) infection remained a major theme in the context of HIV-associated liver disease, although other causes of liver disease garnered increased attention, including fatty liver disease, hepatitis B, and the impact of HIV disease itself on the liver. Although no data from phase III studies of HCV direct-acting antiviral (DAA) drugs for the treatment of HIV/HCV coinfection were presented at CROI 2016, a broad range of HCV DAA–related topics were presented, including accumulating experience with real-world performance of DAA-based regimens outside of clinical trials, drug interactions between DAA and antiretroviral drugs, treatment of acute HCV infection, and retreatment of individuals whose DAA-based regimens failed and those in whom resistance to DAA drugs emerged. A summary of select abstracts from CROI 2016 is presented, including discussion of clinical relevance where appropriate and areas for future research.

Keywords: CROI, 2016, hepatitis, HIV, coinfection, HCV, direct-acting antiviral, DAA, liver, fibrosis, hepatitis C, hepatitis B, hepatitis D, hepatitis E

The HCV Cascade of Care and Improving Access to HCV Treatment

The US Centers for Disease Control and Prevention recommends hepatitis C virus (HCV) screening for all individuals born between 1945 and 1965 (baby boomers).[1] Despite these recommendations, in data presented at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI) from a Detroit cohort of more than 40,000 baby boomers seen in clinics from 2014 to 2015, only 21.3% were tested for HCV infection and 29% of those diagnosed with HCV infection received treatment (Abstract 531). Those with lower income or with Medicaid insurance were less likely to receive HCV treatment, highlighting the disparity in HCV treatment access for low-income individuals. Screening in the emergency department (ED) is one strategy to expand HCV screening to a broader population and did not prolong the length of stay in ED patients who received other lab testing in a California study of ED-based HCV testing (Abstract 532).

One of the challenges of scaling up treatment with direct-acting antiviral (DAA) drugs is the currently limited number of HCV practitioners. The Washington DC–based, observational ASCEND study demonstrated the feasibility of primary care–based HCV treatment with a fixed-dose combination of ledipasvir and sofosbuvir (Abstract 538LB). Three hundred four participants treated attained high rates of sustained virologic response 12 weeks after cessation of therapy (SVR12) across a variety of HCV practitioners; SVR12 rate was 96.7% with primary care physicians, 94.9% with nurse practitioners, and 92.1 % with infectious diseases or hepatology specialists. In the subset of 62 participants coinfected with HIV, SVR rates remained high and did not differ substantially by practitioner type. It is important to note that participants were not randomly assigned to type of treating practitioner; however, fibrosis stage was evenly distributed across treatment groups. Notably, visit adherence was significantly higher among those treated by primary care physicians and nurse practitioners than among those treated by specialists (49%, 51%, and 19.2%, respectively; $P = .002$). This study provides support for community-based, nonspecialist HCV treatment, which can expand access to HCV therapy. A survey of Baltimore primary care physicians indicated that although very few primary care practitioners were prescribing HCV treatment and the majority did not think primary care practitioners should treat HCV infection, 65 % wanted additional HCV training, and those with more than 20% of patients with HCV infection were much more likely to want to prescribe HCV treatment (Abstract 537). Thus, for selected primary care practitioners with a large population of HCV-infected patients, primary care–based HCV treatment may be a viable strategy to expand to the pool of HCV practitioners and the uptake of HCV therapy.

Observational and Real-World Experiences With HCV Infection

Overall, data from CROI 2016 demonstrate robust performance of DAA regimens in "real-world" populations that may differ markedly from populations enrolled in highly selected clinical trials. A US Department of Veterans Affairs database of HCV-monoinfected individuals examined a variety of sofosbuvir-based regimens and demonstrated overall SVR12 rates similar to those attained in clinical trials (Abstract 581). As expected, response rates were lower in those with cirrhosis but, reassuringly, were not negatively impacted by black race, diabetes, or higher baseline HCV RNA level. Similarly,

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2 French databases each reported an overall SVR12 rate of 92% in HIV/HCV-coinfected and HCV-monoinfected individuals with a variety of HCV genotypes treated with all-oral DAA regimens (Abstracts 582 and 583).

The German Hepatitis C Cohort (GECCO), also consisting of HCV-monoinfected and HIV/HCV-coinfected participants with HCV genotypes 1 through 4 receiving all-oral DAA regimens, demonstrated an overall SVR12 rate of 92%, which was not impacted by the presence of cirrhosis, diabetes, or HIV coinfection. Among 283 HIV/HCV-coinfected individuals in GECCO, SVR rates were high (86%-98%) and did not differ substantially by antiretroviral regimens containing dolutegravir, raltegravir, HIV protease inhibitors (PIs), or HIV nonnucleoside analogue reverse transcriptase inhibitors (Abstract 589). Notably, 8 weeks of treatment with ledipasvir and sofosbuvir resulted in an SVR rate of 92% among those selected for shortened therapy, including 26 HIV/HCV-coinfected individuals, for whom data are lacking to date to support 8 weeks of treatment with ledipasvir and sofosbuvir (Abstract 584). Although this is a small number of individuals, these data are reassuring about the potential of 8 weeks of treatment for those with HIV/HCV coinfection, especially after the disappointing SVR12 rate of 76% with 8 weeks of another nonstructural protein 5A (NS5A) inhibitor–based regimen of sofosbuvir and daclatasvir in the previously published ALLY-2 study.[2]

Data on treatment with HCV DAA drugs outside of clinical trials demonstrate excellent responses; however, progress is needed with regard to HCV screening and linkage to care, as well as treatment outside of specialized centers.

In a New York City federally qualified health center (FQHC) cohort, 89 HCV-monoinfected and HIV/HCV-coinfected individuals treated with sofosbuvir-based regimens attained an overall SVR12 rate of 96%, which did not differ between those who used drugs and those who did not (96% and 95%, respectively; $P = .95$); drug use was defined as current opiate substitution therapy, a positive result on a urine toxicology screen, or a history of active drug use by chart review. This report adds to the growing data demonstrating that individuals with active or prior drug use can be effectively treated with DAA regimens (Abstract 585). A Texas clinic serving a similar low-income urban population demonstrated an impressive ramp up of DAA provision from less than 5 per month to an average of 20 or more per month over 1 year, despite limited resources, using a multidisciplinary approach. The overall SVR rate of 76% was limited by a 16% rate of loss to follow up, highlighting the need for support services for vulnerable populations even with well-tolerated all-oral therapy. Notably, those who completed treatment had an SVR12 rate of 90% (Abstract 587). Project INSPIRE (Innovate and Network to Stop HCV and Prevent Complications via Integrating Care, Responding to Needs and Engaging Patients and Providers) in New York City also used a multidisciplinary approach with care coordination to successfully link more than 500 individuals to HCV care and to initiate HCV treatment for nearly 350 individuals in a population enriched for former and current injection drug use (IDU), mental illness, and other comorbidities (Abstract 534).

Drivers of the HCV Epidemic: Injection Drug Use and Sexual Transmission Among HIV-Infected Men Who Have Sex With Men

Globally, IDU is the major risk factor for HCV acquisition.[3] In the setting of the IDU-associated HIV and HCV outbreaks in Indiana, a detailed molecular epidemiologic analysis of the HCV transmission networks was presented (Abstract 149). The analysis was based on testing of 492 samples, including 311 samples of consensus NS5B sequences for genotyping and 281 samples of the hypervariable region 1 (HVR1) in the HCV envelope region (E1E2 envelope glycoprotein complex) analyzed using next-generation sequencing. The HCV genotypes based on NS5B sequences were 1a (72%), 3 (21%), 2 (5%), and 1b (2%), with 3 clusters including a large cluster of genotype 1a. More strikingly, based on HVR1 sequences, mixed or HCV superinfections were found in 20% of samples tested, indicating ongoing exposures and numerous reintroductions of HCV strains into the population.

Several abstracts highlighted the importance of sexual transmission of HCV infection among HIV-infected men who have sex with men (MSM) as an important and often underrecognized driver of the HCV epidemic. In the US-based HIV Outpatient Study (HOPS) from 2011 to 2013, HCV incidence declined among people who inject drugs (PWID) and heterosexual HIV-infected patients. However, the HCV incidence rate remained stable at approximately 1.1% per year among HIV-infected MSM, an important reminder that this population needs ongoing HCV screening as well as counseling about HCV prevention (Abstract 544).

Recent HCV infection (≤2 years) in an Australian cohort of HIV-infected MSM was statistically significantly associated with sexual exposure compared with IDU (adjusted odds ratio [aOR], 9.91; 95% confidence interval [CI], 3.84, 25.59) and a higher number of male sexual partners. Distressingly, almost half (43%) reported they "never" disclosed their HCV serostatus to sexual partners, and 27% were unaware of the potential for HCV reinfection after curative therapy (Abstract 545).

Prevalent HCV infections in a Vancouver, Canada, cohort of HIV-infected and HIV-uninfected MSM were statistically significantly associated with engaging in anal sex without condoms and crystal methamphetamine use (via injection or other routes). The 5 incident HCV infections detected occurred only in HIV-infected MSM, and 4 of these were attributed to sexual contact rather than IDU (Abstract 546). Of

Sexually transmitted HCV infection remains an underrecognized issue among HIV-infected MSM.

interest, a genetic variation in the low-density lipoprotein receptor gene may be associated with genetic susceptibility to sexual (but not parenteral) acquisition of HCV infection, a potential biologic explanation for why some men who have been exposed repeatedly to HCV via sexual contact with MSM remain uninfected (Abstract 547).

HCV Tratment as Prevention and HCV Vaccine Prospects

Treatment of those at highest risk of transmitting HCV, including PWID and HIV-infected MSM, will be key to realizing the potential of DAA-based treatment to curb and ultimately eliminate the HCV epidemic. Modeling data indicate that treating a minimum of 200 to 300 PWID could lead to elimination of the HCV epidemic in British Columbia, Canada; however, treatment must be paired with reinfection efforts to realize this potential (Abstract 533). In a similar vein, data from a Dutch modeling study suggest that treating all HCV/HIV-coinfected MSM will reduce HCV prevalence, but treatment must be linked with reduction in reinfection to lead to HCV elimination (Abstract 536).

Identifying broadly neutralizing antibodies and the epitopes they target may be one approach to identifying better HCV vaccine candidates. Utilizing an ongoing cohort of PWID, researchers from Amsterdam, the Netherlands, isolated and immortalized HCV E1E2–specific B cells from PWID who had repeatedly cleared HCV infection after numerous exposures (Abstract 152). Antibodies from these B-cell cultures were then purified and their epitopes characterized by alanine-scanning mutagenesis. Antibodies isolated from participants who cleared all HCV infections recognized multiple HCV genotypes. Broadly neutralizing antibodies tended to target epitope II plus domain B on envelope glycoprotein E2 (the so-called AR3 epitope) or the AR4 epitope at the interface of envelope glycoproteins E2 and E1. When tested using an HCV pseudoparticle assay, only antibodies to the AR3 epitope displayed broad neutralizing characteristics.

Acute HCV Infection

Acute HCV infection is an important opportunity to identify and treat new HCV infections, preventing subsequent liver damage and breaking the cycle of ongoing transmission. A Spanish observational study highlighted the disproportionate impact acute HCV infection can have on HIV-infected individuals, among whom a diagnosis of acute HCV infection led to a statistically significantly higher risk of hospitalization or death than among those with acute HCV infection who did not have HIV infection (adjusted hazard ratio [aHR], 2.91, for death; 95% CI, 2.38-3.53) (Abstract 590).

In the interferon alfa era, treatment during the first 6 months to 12 months of acute HCV infection was associated with higher cure rates with shorter duration of therapy. However, the efficacy of shortened interferon alfa–free, DAA-based therapy for acute HCV infection remains unknown. Previously reported at the 66th Annual Meeting of the American Association for the Study of Liver Diseases, the first phase of the SWIFT-C (Sofosbuvir-Containing Regimens Without Interferon for Treatment of Acute HCV Infection) study demonstrated a disappointing 41% relapse rate after 8 weeks of treatment with sofosbuvir and weight-based ribavirin given to 17 HIV/HCV-coinfected participants with acute HCV genotype 1 infection.[4] In a pharmacokinetic evaluation of these individuals, median ribavirin level at end of treatment for those who relapsed was 34% lower than for those who attained an SVR12 ($P = .01$), suggesting that inadequate levels of ribavirin may have contributed to the failure of this strategy (Abstract 99). However, for HCV genotype 1 infections, sofosbuvir and ribavirin is no longer recommended for initial treatment, given the superior performance of NS5A-based regimens.[5]

The optimal treatment approach for acute HCV infection in those with HIV infection using DAA drugs remains to be defined.

With regard to the efficacy of NS5A-based treatment for acute HCV infection, 6 weeks of treatment with ledipasvir and sofosbuvir led to an SVR12 rate of 77% among 26 HIV-infected individuals with acute HCV infection (documented infection for < 24 weeks) and HCV genotype 1 or 4 (Abstract 154LB). Four virologic failures (1 of which was a reinfection) occurred in participants with the highest levels of HCV RNA at baseline (> 7.0 $\log_{10}$ IU/mL). This suggests that individuals with acute HCV infection and high baseline viral loads (in this study, ≥9 million IU/mL) may not be candidates for shorter therapy; however, shorter regimens may be feasible for those with lower baseline viral loads. Two of the 3 individuals who experienced virologic relapse each had 1 baseline NS5A resistance-associated variant (RAV) (1 at position 28 and 1 at position 31). It is unclear how these RAVs contributed to the treatment failures, and data were not presented on baseline RAVs from those who attained an SVR12. Even excluding reinfections and loss to follow-up, the 88% SVR12 rate is suboptimal in an era in which SVR12 rates for chronic HCV infection treated for 12 weeks are greater than 95%.[6] Data from an ongoing study of 8 weeks of sofosbuvir and ledipasvir for treatment of acute HCV infection are eagerly awaited.

Interactions Between HIV Antiretroviral and HCV DAA Drugs

Drug interactions between antiretroviral and DAA drugs are a major consideration in HCV treatment and may impact HCV regimen selection for HIV/HCV-coinfected patients.[5] When coadministered with tenofovir disoproxil fumarate (TDF), ledipasvir increases tenofovir levels, as do antiretroviral regimens boosted by ritonavir or cobicistat. Thus, the higher tenofovir concentrations observed with coadministration of ledipasvir and sofosbuvir with TDF-containing, boosted antiretroviral regimens may potentially increase the risk of nephrotoxic effects.

In a Spanish cohort of 225 HIV-infected individuals treated with ledipasvir and sofosbuvir, coadministration of TDF with ritonavir or cobicistat was not associated with statistically significant changes in renal function, with either 12 weeks or 24 weeks of HCV treatment. Five participants had an estimated glomerular filtration rate (eGFR) below 70 mL/min (by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation) during HCV treatment, but none declined below 50 mL/min. Of those with an eGFR below 70 mL/min, 3 were taking boosted antiretroviral regimens, and eGFR returned to above 70 mL/min in all 5 at the cessation of HCV treatment. One participant taking a regimen of cobicistat-boosted elvitegravir, emtricitabine, and TDF discontinued the regimen because of a decline in eGFR from 89 mL/min to 56 mL/min; eGFR after HCV treatment was 85 mL/min (Abstract 452). In a German study of treatment for acute HCV infection, 7 of 26 participants were taking ritonavir- or cobicistat-containing regimens with TDF while receiving HCV treatment with ledipasvir and sofosbuvir; no renal adverse events were reported (Abstract 154LB).

Currently, the regimen of paritaprevir, ritonavir, and ombitasvir plus dasabuvir (PrOD) can be coadministered with HIV regimens containing unboosted integrase strand transfer inhibitors or boosted atazanavir. Data from uninfected volunteers demonstrated a decrease in darunavir trough concentration of approximately 45% when coadministered with PrOD,[7] resulting in the recommendation that this regimen not be coadministered with these HIV medications pending further data. HCV and HIV outcomes as well as pharmacokinetic data from an ongoing phase II/III study of PrOD among HIV/HCV-coinfected individuals, including those taking ritonavir-boosted darunavir, were presented (Abstract 574). Twenty-two participants with suppressed HIV RNA who were taking an antiretroviral regimen of ritonavir-boosted darunavir once daily were randomly assigned to continue the daily regimen or to increase to 600 mg/100 mg twice daily 2 weeks before initiating HCV treatment with PrOD plus ribavirin for 12 weeks. The majority of participants was HCV treatment naive (19/22) and only 3 had cirrhosis. All participants achieved an SVR12. Five participants experienced HIV RNA blips (< 200 copies/mL) during treatment, with no difference between the daily (n = 2) or twice-daily (n = 3) arms. Compared with pretreatment levels, darunavir trough concentrations decreased by 36% and 17%, respectively, in those taking daily and twice-daily treatment. These data suggest that ritonavir-boosted darunavir may be coadministered with PrOD. More data will be available on completion of the phase III portions of the study; however, the population for which data are most needed, those taking darunavir twice daily who have a history of HIV PI–resistance associated mutations, was not addressed in this study.

Two abstracts described drug interactions of 2 investigational HCV regimens: 1) sofosbuvir and the investigational pangenotypic NS5A inhibitor velpatasvir (Abstract 100), and 2) the investigational NS3 PI ABT-493 and the investigational NS5A inhibitor ABT-530 (Abstract 453). In a study of uninfected volunteers, the impact of ritonavir- or cobicistat-boosted regimens when given with sofosbuvir and velpatasvir was evaluated (Abstract 100). Modest increases in the area under the curve (AUC) of sofosbuvir (< 50%) were observed when coadministered with cobicistat or with ritonavir-boosted atazanavir; large increases in the AUC (2.0-2.5 fold) and maximum plasma concentration C_{max} (4 fold) of velpatasvir were observed when coadministered with ritonavir-boosted atazanavir. Conversely, a roughly 50% increase in the AUC, C_{max}, and minimum plasma concentration C_{min} of tenofovir was observed when given as TDF in combination with cobicistat or ritonavir. The investigators concluded that the data support coadministration of any of these antiretroviral regimens with sofosbuvir and velpatasvir. Pending data from phase III studies, the increase in levels of tenofovir exposure observed when given as TDF in combination with sofosbuvir and velpatasvir is similar to that seen with sofosbuvir and ledipasvir, suggesting that enhanced monitoring for tenofovir toxic effects during coadministration is warranted.

Interactions between the investigational regimen of ABT-493 and ABT-530 and rilpivirine or raltegravir were evaluated in uninfected volunteers (Abstract 453). As expected, given the neutral impact of rilpivirine or raltegravir on coadministered drugs, no substantial changes in levels of ABT-493 or ABT-530 exposure were observed (< 15% change). An approximately 2-fold increase in rilpivirine exposure was observed, although trough levels of raltegravir increased approximately 2.5 fold with a large degree of variability. Overall, the changes identified were similar in magnitude to drugs currently safely coadministered with rilpivirine or raltegravir, suggesting that ABT-493 and ABT-530 can also be coadministered with these drugs.

Resistance-Associated Variants and HCV Retreatment

Clinical data show that the impact of NS5A RAVs is quite different for HCV genotype 1a than for HCV genotype 1b.[8] In a series of in vitro experiments, Newton and colleagues (Abstract 578) explored polymorphic site 28 in NS5A and its impact on other NS5A RAVs. Using a replicon system with genotype 1a or 1b NS5A sequences, the consensus amino acid at position 28 (methionine [M] in 1a; leucine [L] in 1b) was swapped (M28L in 1a, and L28M in 1b) and its impact on fold changes in susceptibility to the NS5A inhibitors daclatasvir, ledipasvir, and ombitasvir in conjunction with other RAVs at positions Q30, L31, and Y93 were assessed. Changing the consensus amino acid at position 28 had little impact by itself on either NS5A background, although the M28L in genotype 1a did result in consistent decreases in inhibitor half maximal effective concentrations (ie, hypersusceptibility). Changes in M or L at position 28 did not have a measurable effect on replication capacity in either background. The M28L variant in genotype 1a substantially lessened the impact (a 10× to >1000× decrease in half maximal inhibitory concentration) of NS5A RAVs Q30E/H/K/R, L31M, and Y93H, essentially making the genotype 1a replicon behave more like genotype 1b with respect to the impact of these RAVs

on daclatasvir, ledipasvir, and ombitasvir. The converse was also true: introducing the L28M variant in genotype 1b substantially increased resistance to daclatasvir, ledipasvir, and ombitasvir for RAVs L31M and Y93H.

In a survey of NS5A RAVs from 973 HCV samples submitted for testing, including 776 with genotype 1a and 197 with genotype 1b, resistant variants were identified in 39.6% of 1a and 43.1% of 1b isolates (Abstract 579). RAVs at positions 28, 30, 31, 58, and 93 were counted when present in more than 10% of the viral population on deep sequencing using the Illumina MiSeq platform. Unfortunately, clinical data were not available for the samples (eg, whether the individuals were DAA treatment experienced or naive). Given the nature of the RAVs identified and the percentages with multiple RAVs (40% of genotype 1a, and 26% of genotype 1b), it is likely a substantial proportion were DAA treatment failures exposed to an NS5A inhibitor. The most prevalent RAVs in genotype 1a were at position Q30 and Y93, and in genotype 1b the specific Y93H RAV was most prevalent. Susceptibilities of the various RAVs to daclatasvir, ledipasvir, and ombitasvir (in replicons or using participant-derived NS5A sequences) in genotypes 1a and 1b backgrounds were tested and, in line with previous results, showed larger shifts in activity ("more" resistance) in genotype 1a than in 1b replicons, particularly at position 93. Multiple RAVs present in genotype 1a also tended to cause higher-level resistance than any single RAV.

Baseline NS5A RAVs impact responses to NS5A-containing DAA regimens, particularly in populations with other predictors of a poorer treatment response, such as prior treatment failure and cirrhosis. Several studies evaluated the impact of baseline RAVs on treatment outcomes in clinical trial and real-world settings. Fourati and colleagues conducted a survey of baseline N5SA and NS5B RAVs in 177 individuals and their impact on treatment outcomes with 12 weeks of sofosbuvir and daclatasvir (Abstract 577). The presence of RAVs at key NS5A (ie, 28, 29, 30, 31, 32, 58, 62, 92, and 93) and NS5B (ie, 159, 282, 316, 320, and 321) positions was assessed using population sequencing. Individuals were infected with HCV genotype 1a (n = 44), 1b (63), 3 (29), or 4 (41) and were treatment naive or had previously taken peginterferon alfa and ribavirin, with or without an HCV PI. Cirrhosis was present in 44%, including 55% of those with HCV genotype 3 infection. Baseline NS5A RAVs were identified in 9% of those with genotype 1a (M28V/T and Q30R) and 21% of those with genotype 3 (A30K/S, A62L, and Y93H). No S282T NS5B RAVs were detected at baseline. Only 8 of the 177 individuals experienced virologic failure, and having genotype 3 (5/8) or cirrhosis (7/8) were the most common characteristics associated with treatment failure. There was a trend toward an association between having the Y93H variant at baseline and virologic failure (96% and 75% SVR12 rates for those without and with Y93H, respectively; $P = .06$). Importantly, the regimen used, sofosbuvir and daclatasvir without ribavirin, is not recommended for individuals with HCV genotype 3 infection who have cirrhosis.[5]

In terms of predictors of DAA treatment failure, 13 individuals who experienced virologic failure after treatment with ledipasvir and sofosbuvir in a cohort at Mount Sinai Medical Center in New York, New York, were evaluated; 8 (62%) had cirrhosis, 5 (38%) had a history of hepatocellular carcinoma (HCC), and 6 (46%) had a prior treatment failure with an interferon alfa–based regimen (Abstract 588). Black race (OR, 4.96; 95% CI, 1.18-13.09) and male sex (OR, 4.62; 95% CI, 1.28-16.6) were statistically significantly associated with treatment failure, as has been observed in other real-world cohorts that have reported treatment failures associated with black race and male sex.[9,10] Baseline NS5A resistance data were not available, but as expected, the majority of individuals (15/17) who underwent resistance testing after virologic failure had 1 or more NS5A RAVs.

In another study that assessed the impact of baseline RAVs on treatment response, NS3 and NS5A RAVs were assessed retrospectively in interferon alfa–experienced participants with HCV genotype 1a infection treated with PrOD and ribavirin in the SAPPHIRE-II and TURQUOISE-II studies, as well as those with genotype 1b infection treated with PrOD without ribavirin in the PEARL-II and TURQUOISE-III studies (Abstract 539LB). Various RAV definitions (eg, drug class RAVs vs drug-specific RAVs) were assessed using next-generation sequencing and reported at various thresholds (1%-15%); ombitasvir-specific NS5A RAVs were present in 12% of participants with genotype 1a at the 15% threshold. In this analysis, baseline ombitasvir RAVs had no substantial impact on treatment of those with genotype 1a with PrOD plus RBV (95% and 97%, for those with and without RAVs, respectively). Baseline NS3 RAVs or RAVs in those with genotype 1b also did not impact treatment response. Unfortunately, the group of most clinical interest with the highest likelihood of being impacted by baseline RAVs, those with genotype 1a treated without ribavirin (in the PEARL-IV study), were omitted from this analysis.

The impact of HCV RAVs on responses to DAA-based treatment cannot be assessed in a vacuum. Regimen and patient characteristics also have a substantial impact on treatment response in addition to consideration of RAVs.

The phase III ALLY-2 study evaluated treatment with sofosbuvir and daclatasvir for 12 weeks or 8 weeks in HIV/HCV-coinfected participants.[2] In a follow-up study, the presence of NS5A or NS5B RAVs at baseline and treatment failure were assessed using next-generation sequencing to determine if RAVs missed by population sequencing appeared to impact clinical responses (Abstract 575). For 14 participants who experienced virologic failure (3 with 12 weeks and 11 with 8 weeks of treatment with sofosbuvir and daclatasvir), samples taken at baseline and at treatment failure were analyzed using the Illumina MiSeq platform. A 1% detection threshold was used to identify RAVs in NS5A and NS5B. In participants for whom 12 weeks of therapy failed, next-generation sequencing did not detect additional NS5A

RAVs compared with population sequencing. In 3 of 11 participants for whom 8 weeks of therapy failed, additional NS5A RAVs were detected with next-generation sequencing at time of failure; however, all were at low prevalence (< 2 %) by week 24 of follow-up. No minor variants of clinical significance (eg, S282T) were found with next-generation sequencing in NS5B. Overall, these data support the emerging theme that population sequencing identifies the vast majority of clinically significant NS5A RAVs.

After failure of a DAA regimen, treatment-emergent RAVs may be selected, and the impact of these RAVs on retreatment may be different than that of baseline RAVs. Two studies of RAVs and retreatment response were presented. Wilson and colleagues presented a detailed analysis of RAVs and SVR outcomes for individuals retreated with a fixed-dose combination of sofosbuvir and ledipasvir for 12 weeks following failed short-course therapy (4-6 weeks) with this regimen plus 1 to 2 additional agents (Abstract 580). The investigators first examined whether baseline NS3 and NS5A RAVs impacted treatment response to the initial regimen. With 8 weeks of treatment consisting of sofosbuvir and ledipasvir plus the investigational NS3 PI GS-9451, baseline RAVs did not have a clear impact on treatment response, although there was limited power to detect a substantial impact based on the small numbers. Individuals with baseline NS5A RAVs did have a numerically lower response rate to 6 weeks of sofosbuvir and ledipasvir plus GS-9451 (60% [3/5] of those with NS5A RAVs vs 85% [17/20] of those without NS5A RAVs). SVR rates in those who received 4 weeks of treatment with sofosbuvir and ledipasvir plus GS-9451 or sofosbuvir and ledipasvir plus GS-9451 and GS-9669 (an investigational thumb site II nonnucleoside inhibitor of NS5B) were low overall (40% and 20%, respectively), despite a treatment-naive population with Metavir fibrosis stages F0 to F2. Thirty-four individuals (33 treatment failures at 4 weeks, and 1 at 6 weeks) were retreated with sofosbuvir and ledipasvir for 12 weeks. Before retreatment, 29 had NS5A resistance, including 28 with greater than 100 times ledipasvir resistance. The SVR rate was 90% (26/29) in those with NS5A RAVs and 100% (5/5) in those without NS5A RAVs.

The second study reported retreatment results for 9 participants whose treatment failed in the ION-4 study (sofosbuvir and ledipasvir for 12 weeks)[6] who were then re-treated with sofosbuvir and ledipasvir plus ribavirin for 24 weeks (Abstract 573). All 9 participants were black and had the IL28B non-CC genotype, 7 had HCV genotype 1a infection, and 7 were men. Only 2 participants had cirrhosis, and 7 were taking efavirenz. Participants were retreated quickly, with a mean time from treatment failure of 43 days; despite this, baseline HCV RNA level was 6.4 $\log_{10}$ IU/mL. Kinetics of viral suppression during treatment did not appear to be impacted by prior treatment failure; 100% of participants had an HCV RNA level below the lower limit of quantification at week 4, and this was maintained through the end of treatment. One participant experienced virologic failure, with relapse 4 weeks after cessation of treatment. The SVR12 rate was 89% (8/9). RAVs associated with high-fold reduced susceptibility to ledipasvir (L31M + H58D, Y93N/H, or L31M/V) were present in more than 99% of the quasispecies in 7 of 9 participants but did not have a clear impact on retreatment response (6/7; 86% with an SVR). In the lone participant who relapsed, L31M was present at retreatment and retreatment failure.

A second abstract related to the ION-4 study presented data from a genome-wide association study undertaken to identify previously unrecognized host genomic determinants that may have contributed to treatment failure (Abstract 601). Ultimately, no notable genome-wide associations with treatment failure were found.

Collectively, these studies highlight that the impact of RAVs, at baseline or that emerged during treatment, on response to HCV treatment cannot be assessed in a vacuum. Individual and regimen characteristics have a substantial impact on treatment response, making it all the more difficult to assess the specific impact of RAVs on treatment outcomes.

Life After HCV Cure: Fibrosis Regression and Morbidity After an SVR12

HCV cure is associated with improved morbidity and mortality in those who attain an SVR12; however, a subset will still experience hepatic complications despite cure, including fibrosis progression and HCC.[11] In a Spanish cohort of HIV-infected individuals with cirrhosis who were cured of their HCV infection with DAA-based therapy, the majority showed improvement or no change in liver function at the time of SVR12, as measured by Child-–Turcotte-Pugh (CTP) or Model for End-Stage Liver Disease (MELD) score. However, a subset demonstrated worsening liver function despite HCV cure, with 8% having worsening MELD scores and 27% having worsening CTP scores (Abstract 603).

In a French cohort of 235 HIV-infected individuals successfully treated for HCV infection, hepatic complications were rare but did occur in 2 individuals who developed hepatic decompensation within the first year after cure (a rate of 3.9/1000 person-years) (Abstract 605). Focusing on 38 individuals with HCC who had been previously cured of HCV infection, 29% to 38% did not have cirrhosis, depending on the methodology used, suggesting that risk for hepatic cancer remains in some individuals despite HCV cure and may not always be associated with the presence of cirrhosis (Abstract 604). Overall, these data serve as a reminder that individuals with cirrhosis should continue to be screened for HCC even after HCV cure, as the risk for hepatic cancer may remain even if fibrosis regresses.

Data from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) cohort highlighted the impact of factors known to be associated with liver disease in those with HIV infection (Abstract 150). The cohort included 34,044 HIV-infected adults, including a subset of 12,158 with at-risk alcohol use as measured at entry into the study. Adjusted population attributable risk fractions (aPAFs) were determined, taking into account the prevalence of a given risk factor along with its aHR for end-stage liver disease

(ESLD). In the entire cohort, HCV infection accounted for 33% of ESLD (aPAF), and a CD4+ cell count below 200/µL and hepatitis B virus (HBV) coinfection had aPAFs of 25% and 16%, respectively. In the subgroup for whom alcohol use data were available, the contribution of HCV infection to ESLD increased slightly to 35%, and alcohol use itself accounted for 33% of ESLD. These data support the profound positive impact widespread HCV treatment could have on ESLD incidence in those with HIV infection, with or without alcohol use.

Impact of HCV Infection Outside the Liver

Hepatitis C infection has been associated with a number of extrahepatic manifestations, including cryoglobulinemia, B-cell lymphoma, diabetes mellitus, and kidney disease.[12] Further, successful eradication of HCV can lead to improvement or resolution of these conditions.[13,14] Several abstracts examined the impact of DAA treatment on extrahepatic manifestations of HCV infection in those with HIV coinfection.

Two abstracts examined the impact of DAA therapy on diabetes. In a detailed analysis of 29 HCV–infected individuals (10 coinfected with HIV) treated with DAA drugs, levels of fasting glucose and hemoglobin A_{1C} improved rapidly during therapy (Abstract 610), with mean reductions of 53 mg/dL and 1.95%, respectively. Of 25 individuals with evaluable end point data, 6 of 25 (24%) individuals required a dose reduction of insulin or metformin. In a large-scale analysis of the Spanish AIDS Study Group (GeSIDA) cohort (SVR, n = 633; no SVR, n = 992), after controlling for other factors, an SVR following HCV treatment was associated with a reduced incidence of renal events (HR, 0.38; $P = .046$) and diabetes mellitus (HR, 0.56; $P = .018$) (Abstract 611). As expected, SVR was also associated with a decreased risk of overall and liver-related mortality.

An analysis from the Swiss HIV Cohort Study examined the impact of HCV seropositivity and HCV viremia on a number of outcomes, including renal disease and liver-related and all-cause mortality (Abstract 612). All HCV-seropositive individuals (regardless of viremia) had an increased incidence ratio for liver-related death compared with HCV-seronegative matched controls, (although viremia tended to further increase the incidence ratio). In contrast, incidence ratios for liver disease were only statistically significantly elevated in those with HCV viremia. Non–liver-related mortality was not associated with HCV seropositivity.

Frailty and low muscle mass are increasingly being evaluated as potential complications of long-standing HIV infection and premature aging. The potential contributions of viral hepatitis coinfection (HCV or HBV) to low muscle mass were evaluated in the MACS (Multicenter AIDS Cohort Study) and WHIS (Women's Interagency HIV Study) cohorts (Abstract 609). In this cross-sectional study, low muscle mass was found in 27% of coinfected participants versus 12% of HIV-infected and 10% of uninfected controls. Coinfected participants were more likely to have low muscle mass (OR, 1.94) than HIV-monoinfected participants. Among coinfected participants, lack of HIV suppression was statistically significantly associated with low muscle mass (OR, 2.28; 95% CI, 1.11-4.66). The impact of successful HCV therapy on low muscle mass should be evaluated in future studies.

Progression of Liver Fibrosis Among Those With HIV Infection: Assessment, Pathogenesis, and Interventions

Liver Fibrosis: Assessment

Noninvasive methods for staging of liver fibrosis have largely replaced biopsy in the management of HCV infection, and staging of fibrosis is essential for obtaining health insurance approval of HCV medications in the United States. Two abstracts (Abstracts 527 and 528) compared various methods for staging of liver fibrosis in HIV/HCV-coinfected individuals. Fibrosis-4 (FIB-4) and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) scoring are readily available indices used to stage liver disease, and FibroTest (or FibroSure) is a commercially available blood test that is also used to stage liver disease in the setting of HCV infection. The variability between these 3 methods, when normalized to a 4-point scale, was assessed in the setting of the phase IV ASCEND trial of HCV treatment in a community-based setting (Abstract 528). There was consistently less variability between APRI and FIB-4 scores than between FibroTest results and FIB-4 or APRI score, which is unsurprising as these metrics rely on many of the same laboratory values (AST level, platelet count). When compared across liver disease stages, less variability between FibroTest results and FIB-4 or APRI score was observed at estimated early stage disease. The absolute values are lower, so less variability would be expected. Coinfection status did not appear to impact the variability. It is tempting to speculate that the differences correlate to a better predictive ability of one test versus the others; however, this study did not evaluate correlations to fibrosis stage as assessed by another method (eg, biopsy or transient elastography) or outcomes.

FIB-4 has been shown in cohort studies to be a good predictor of HCV liver disease–related outcomes in coinfected individuals.[15] Transient elastography is an ultrasound procedure that can be performed at the bedside and that stages liver disease based on liver stiffness; although cutoffs vary, 9.5 kPa and 12.5 kPa are often used to indicate Metavir stages 3 and 4 liver disease, respectively.[16,17] In a retrospective analysis, 1159 HIV/HCV-coinfected individuals were assessed from first transient elastography measurement until last follow-up for liver-related events (decompensation, HCC) or death (Abstract 527). Associations between outcomes were assessed, including a comparison of first FIB-4 score and liver stiffness. Liver-related events occurred in 75 participants (6.5%) over a median follow-up period of 5.8 years. Those who experienced liver-related events were more likely to ingest more than 50.0 g of alcohol per day, to have a CD4+ cell count below 350/µL, and to not have achieved an SVR with HCV therapy. Not surprisingly, death was more common in

those with liver-related events. Both baseline FIB-4 score and liver stiffness each predicted liver-related events or death: mean baseline FIB-4 scores were 3.14 and 1.24 and mean liver stiffness measurements were 26.0 kPa and 8.0 kPa for those with and without liver-related events, respectively. However, the area under the receiver operating characteristic curve (AUROC) for transient elastography as a predictor of liver-related events or death was significantly higher than for FIB-4 scoring ($P < .001$). Using cutoffs of a FIB-4 score of 3.25 or higher and a liver stiffness measurement of 9.5 kPa or higher, the aHRs for liver-related events were 5.36 (3.22-8.93) and 18.7 (9.0-38.7), respectively

A unique aspect of transient elastography is that, rather than a binary designation of cirrhosis or no cirrhosis, its values are continuous variables as liver stiffness increases from the 12.5 kPa to 14.6 kPa threshold for cirrhosis to the maximum value possible of 75.0 kPa. Merchante and colleagues from Spain presented additional data from the prospective HEPAVIR study cohort on the ability of a liver stiffness value of 21.1 kPa to differentiate individuals at risk for variceal hemorrhage (Abstract 530). The cohort consists of 488 HIV/HCV-coinfected participants, predominantly men, most taking antiretroviral therapy (92%), with a diagnosis of cirrhosis determined by a liver stiffness measurement of greater than 14.0 kPa and no prior decompensation. At entry into the study, 90% of individuals had CTP class A and 10% had CTP class B cirrhosis. Prior to 2009, all participants underwent screening for esophageal varices with esophagogastroduodenoscopy (EGD), and only those with a liver stiffness measurement greater than 21.0 kPa underwent EGD screening after 2009. The primary end point was time to first instance of variceal bleeding, with a median follow-up period of 53 months. Consistent with prior results, no participant who maintained a liver stiffness measurement of less than 21.0 kPa suffered from variceal bleeding during the follow-up period (n = 128), for a 100% negative predictive value. The probability of variceal bleeding was significantly higher in those with a baseline liver stiffness measurement of greater than 21.0 kPa ($P = .001$), and 3.2% of patients with a liver stiffness measurement of greater than 21.0 kPa experienced variceal bleeding. Six of 16 patients with variceal bleeding died. In a second study from the same group, a combination of liver stiffness measurement and CTP scoring achieved better prediction of liver decompensation events than either alone (Abstract 529). Despite this, CTP class A cirrhosis was always predictive of a lower rate of liver decompensation than was CTP class B cirrhosis; liver stiffness measurement was used to further separate risk among those within a given CTP class.

Liver Fibrosis: Pathogenesis and Interventions to Slow Progression

Liver disease remains a major cause of morbidity and mortality among those with HIV infection.[18] Although much attention continues to be focused on viral hepatitis coinfection, other factors such as fatty liver disease and HIV infection itself are increasingly recognized as contributing to liver disease among those with HIV infection.

Despite the identification of many factors epidemiologically linked to HCV-associated liver disease progression, the ability to predict which individuals will progress and how fast the progression will be remains limited. In an analysis of the ALIVE (AIDS Linked to the Intravenous Experience) study cohort, semiannual transient elastography assessments were used to examine factors associated with the progression of liver disease and progression to cirrhosis (Abstract 548). Over 8 years of follow-up, 10% of participants progressed from having no or low fibrosis (< 8.0 kPa) to having cirrhosis (> 12.3 kPa), and as expected, moderate or severe fibrosis was associated with a higher rate of mortality. Although changes in transient elastography scores during semiannual visits effectively determined if liver disease had progressed, a high false-positive rate is likely to limit its clinical usefulness. The investigators concluded that accurate prediction of liver disease progression was imperfect and that withholding treatment from those less likely to progress was not justified.

HCV genotype 6 infection is endemic in Southeast Asia and was associated with significantly more fibrosis progression (measured by transient elastography) than genotype 1 infection (aOR, 4.02; $P = .047$) in a cohort of untreated HIV/HCV-coinfected individuals in Thailand followed up for a median of 2.1 years. These data suggest that those with HCV genotype 6 infection should be prioritized for treatment in resource-constrained settings, given the risk for disease progression (Abstract 606).

Successful treatment of HCV infection remains one of the most powerful interventions to prevent progression of liver disease in those with HIV/HCV coinfection.

Progression of liver fibrosis related to HCV infection is accelerated in those coinfected with HIV, particularly if HIV replication is not controlled.[19] However, the effects of HIV replication and CD4+ T-cell depletion on liver fibrosis progression, independent of viral hepatitis coinfection, have not been well characterized. Data on 14,198 HIV-infected participants from the Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort were used to evaluate the contribution of various factors to progression of liver fibrosis measured by FIB-4 scoring (Abstract 558). Progression was defined as an increase in FIB-4 score from below 1.45 to above 3.25 (advanced fibrosis) during the follow-up period. Of participants, 1386 (9.7%) progressed to advanced fibrosis over a median of 3 years. Although classic factors such as HCV or HBV coinfection and alcohol use were associated with progression of fibrosis, HIV viral load and CD4+ cell count were also statistically significantly associated with progression of fibrosis. Increased risk for progression with higher HIV viral load or lower CD4+ cell count as well as statistically significant interaction between having an HIV

RNA level above 500 copies/mL (aHR, 1.7) and a CD4+ cell count below 200/µL (aHR, 3.3) further strengthened the findings when combined (aHR, 7.3; 95% CI, 6.4-8.3). These results lend further support to the recommendation to treat all HIV-infected individuals at diagnosis. A cohort study from Germany with smaller numbers but liver fibrosis assessed by transient elastography reported similar results (Abstract 560). In this cohort of 432 HIV-infected individuals, only 16% of whom were coinfected with HCV, uncontrolled HIV replication was associated with development of severe liver fibrosis (≥12.5 kPa; HR, 2.43; $P = .034$).

In a complementary study, the intrahepatic effects of HIV therapy on HCV infection were evaluated using single-cell laser microdissection to assess intrahepatic HCV RNA levels in individual hepatocytes. Specifically, Quinn and colleagues set out to determine what effect antiretroviral therapy has on the intrahepatic HCV viroscape and to identify potential effector mechanisms for any effect seen (Abstract 151). The investigators were able to show that the number of HCV RNA–containing hepatocytes correlated positively with plasma HCV viral load and that following antiretroviral therapy the number of HCV RNA–containing hepatocytes decreased. The amount of HCV RNA per cell did not seem to change with antiretroviral therapy. Intrahepatic gene expression related to type I interferons (IFI16) and antigen presentation (HLA-E, CIITA) did increase and correlated with decreases in HCV RNA set points.

Two cohort studies demonstrated the protective effect of statins on progression of liver disease. In a retrospective cohort study of HIV/HCV-coinfected individuals in the US Department of Veterans Affairs Clinical Case Registry, use of statins more than 30% of the time during the evaluable time period protected against development of cirrhosis, particularly in those with an alanine aminotransferase (ALT) level below 40 IU/L (Abstract 550).

In a study of HCV-monoinfected US veterans that examined differences between the specific statins used, atorvastatin and fluvastatin were associated with larger decreases in FIB-4 score over time compared with other statins (Abstract 551). For all statins combined, a cumulative defined daily dose (cDDD) of greater than 180 was associated with an HR of 0.6 for cirrhosis and 0.51 for HCC, indicating that longer exposure to statins was associated with a dose-dependent reduction in development of cirrhosis and liver cancer.

In another study involving statins, researchers examined the role of statins on nonalcoholic fatty liver disease—defined as a liver-to-spleen attenuation value of less than 1.0 measured by computed tomography scan—in an exploratory secondary analysis of a clinical trial that examined the impact of atorvastatin on subclinical coronary artery disease.[20] Of 40 HIV-infected individuals, 9 with nonalcoholic fatty liver disease and 28 without, 40 mg daily of atorvastatin was associated with a significant increase in liver-to-spleen attenuation ratio, suggesting a decrease in fatty liver, compared with placebo (0.46 vs -0.4, respectively; $P = .03$). The change in hepatosteatosis was statistically significantly correlated with decreases in low-density lipoprotein cholesterol, suggesting and association between statin use and reduced lipid levels (Abstract 553).

A number of epidemiologic studies have noted that coffee consumption may have protective effects against the development of liver fibrosis and a variety of other conditions.[21-23] In a study from the French Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS) CO13 HEPAVIH cohort, the impact of coffee consumption on mortality was investigated in HIV/HCV-coinfected participants (Abstract 549). The study included 1035 HIV/HCV-coinfected participants for whom a baseline questionnaire was available and vital status was recorded for at least 1 follow-up time point. The median follow-up period was 5 years, and 77 deaths occurred of which 43% were HCV-related. Coffee consumption of 3 or more cups per day was associated with a decreased risk of death (aHR, 0.5; 95% CI, 0.3-1.0; $P = .045$). However, being treated for and cured of HCV infection had the most profound effect (aHR, 0.2).

Epidemiologic data continue to support the protective effect of coffee consumption and statin use on progression of liver disease, including among those with HIV/HCV coinfection.

IDU is the major risk factor for HCV acquisition, and HCV infection and IDU can lead to systemic immune activation, yet the relative contribution of each is poorly understood. Peripheral markers of inflammation were measured among 4 groups: 1) those actively injecting heroin, 2) those with previous IDU who had not injected heroin for 1 month to 2 months, 3) those with previous IDU who had not injected heroin for 3 months to 4 months, and 4) those who did not inject heroin. The investigators assessed the relative contributions of IDU and HCV infection (50%-60% of those who had injected heroin at some point had detectable HCV RNA) to systemic inflammation (Abstract 597). Active IDU and HCV viremia each contributed to CD4+ and CD8 T-cell activation. Levels of tumor necrosis factor–α and soluble CD14 decreased and became comparable to those observed in uninfected controls in HCV aviremic individuals who ceased injecting heroin. This shows that both IDU and HCV viremia contribute to immune activation, although more work is needed to better define their individual roles.

Two abstracts evaluated the impact of HCV DAA therapy on immune activation and intrahepatic immune response on paired liver biopsies. Using paired liver biopsies from individuals treated with sofosbuvir plus ribavirin in the SPARE study, Orr and colleagues utilized quantitative image analysis to determine changes in intrahepatic immune cells or activation (Abstract 600). The number of intrahepatic CD8 T cells decreased in all areas (parenchyma and portal) after therapy, regardless of outcome (SVR vs virologic relapse). The number of CD4+ cells also decreased but only in portal areas. Similarly, the number of activated peripheral CD4+ T cells decreased during therapy with DAA drugs, and this decrease mirrored decreases in ALT levels (Abstract 599). In this second

study, paired liver biopsies showed substantial decreases in histology activity index inflammation scores posttherapy, with one group also showing a substantial decrease in fibrosis after completion of 3 months of therapy.

Hepatitis B: Testing, Transmission, and Treatment

Hepatitis B infection is an important driver of morbidity and mortality among HIV-infected individuals worldwide. Uptake of HBV testing remains low in many African HIV clinics, with testing increasing only slightly from 10.4% in 2010 to 23% in 2012 in urban HIV clinics in 9 African countries participating in the International Epidemiologic Databases to Evaluate AIDS (IeDEA), emphasizing the importance of increasing the availability of point-of-care rapid hepatitis B surface antigen (HBsAg) tests. More than 90% of HBV-infected individuals received tenofovir-based antiretroviral therapy, which is preferred for those with HBV coinfection because of its efficacy as HBV treatment (Abstract 565).

Access to tenofovir for HBV/HIV-coinfected individuals was lower in a West African study, ranging from 15% to 61% depending on the locale (Abstract 568). In a Kenyan cohort of HIV-infected individuals, prevalence of HBsAg was 6.3% and was an independent predictor of early mortality (aHR, 1.84, for death; 95% CI, 1.3-2.6). Initiation of tenofovir-based antiretroviral therapy appeared to mitigate this risk (aHR, 1.45, for death; 95% CI, 0.0-2.2), whereas the risk of death remained statistically significantly elevated among those who tested positive for HBsAg who initiated antiretroviral therapy that did not contain tenofovir (aHR, 3.32, for death; 95% CI, 1.8-6.2) (Abstract 562). Initiation of antiretroviral therapy was also associated with regression of fibrosis in a majority of HIV/HBV-coinfected individuals in Nigeria, 81% of whom initiated therapy with a tenofovir-containing regimen (Abstract 564).

Tenofovir-based HIV regimens are a crucial part of optimal treatment of HIV/HBV-coinfected individuals. Lack of appropriate HBV screening and limited access to tenofovir disoproxil fumarate affect certain high-prevalence areas such as Africa.

In the Swiss HIV Cohort Study, the incidence of HCC increased among HIV/HBV-coinfected participants with each year not on TDF (adjusted incidence rate ratio [aIRR], 1.13; 95% CI, 1.08-1.19), and HCC incidence was highest among those not on TDF for more than 4 years (aIRR, 4.04; 95% CI, 2.1-7.77). These data emphasize the importance of tenofovir in the treatment of HIV/HBV coinfection (Abstract 566).

In sub-Saharan Africa, HBV is thought to be primarily transmitted during childhood. However, data from the Rakai Community Cohort Study demonstrated incident HBV infection in HIV-infected adults, particularly those aged 15 years to 29 years, indicating additional opportunities for vaccination beyond childhood to prevent HBV transmission (Abstract 563). Only 40% of HIV-infected children from 6 Asian countries had a history of HBV vaccination, and more than three-quarters had no protective HBsAb, regardless of vaccination status, emphasizing the need for improved vaccine coverage and revaccination in those for whom the initial series was not effective (Abstract 860).

Several abstracts demonstrated the protective effect of HBV-active antiretroviral regimens against incident HBV infection. In the Rakai Community Cohort Study, lamivudine-based antiretroviral therapy was associated with a 76% reduced risk of HBV acquisition compared with no antiretroviral therapy ($P = .001$), and no new infections occurred in individuals taking a tenofovir-based antiretroviral regimen (Abstract 563). In the Swiss HIV Cohort Study, dually HBV-active antiretroviral therapy that contained tenofovir and lamivudine or emtricitabine reduced HBV incidence substantially (aHR, 0.3; 95% CI, 0.2-0.5) compared with nondually HBV-active therapy (Abstract 567). Collectively, these data emphasize the importance of vaccination and screening for HBV among HIV-infected individuals and of ensuring access to tenofovir-based antiretroviral therapy in those identified with HBV coinfection.

Hepatitis A, D, and E

Hepatitis A

Vaccination against hepatitis A virus (HAV) infection is recommended for all HIV-infected MSM as well as other high-risk populations[24]; despite this, limited data are available on immunity and compliance with vaccination recommendations. A cross-sectional analysis conducted at several time points within the Medical Monitoring Project evaluated baseline and new HAV immunity in a population of 18,095 HIV-infected individuals (Abstract 591). At baseline, 55% of the population had evidence of HAV immunity, including 57% of 8234 MSM. Over the time period evaluated, from 2009 to 2012, 15% of the population showed evidence of newly acquired HAV immunity (either documentation of vaccination or new anti-HAV antibodies not present at baseline). Of the 360 individuals with documented vaccination, factors that were associated with vaccination included younger age (18-29 years), less than 5 years since HIV diagnosis, detectable HIV viral load, and screening for sexually transmitted infections within the last 12 months. At the end of the study period, 38% of individuals lacked evidence of HAV immunity or vaccination, including 36% of HIV-infected MSM. More work is needed to improve vaccination rates in high-risk populations such as HIV-infected MSM.

Hepatitis E

Hepatitis E virus (HEV) infection is endemic in many parts of the world, including India and Africa, and is now estimated to be the leading cause of acute viral hepatitis worldwide.[25] Two studies presented at CROI 2016 examined HEV seroprevalence and HEV RNA positivity in cohorts from the United States (Abstract 593) and Uganda (Abstract 594). HEV infection is not considered endemic in the United States, and

recent studies have found a seroprevalence of 6% that appears to be decreasing.[26] In a retrospective analysis of 2919 stored plasma samples from HIV-infected men (313 samples) and women (2606 samples) from the MACS and WIHS cohorts, HEV RNA assays were performed to assess for acute or chronic HEV infection. Numerous samples were available for patients over time, and the samples were selected for testing based on "biomarkers of liver disease and immune suppression," which were not strictly defined but are assumed to be elevated ALT and AST levels and CD4+ cell counts. Only 3 of 2919 samples tested positive for HEV RNA, with 2 cases being consistent with acute, self-limited HEV infection. The third case did appear to be chronic HEV infection, with consistently detectable HEV RNA over a period of 3 years. This case was unusual, given a CD4+ cell count above 200/µL; however, there was a clear trend of decreasing CD4+ cell count over the observation period, with dips below 200/µL. Although HIV RNA levels were not reported, it seems likely this individual was not taking antiretroviral therapy and had a substantial degree of immunosuppression. Supporting the known epidemiology of HEV disease in the United States, all isolates were genotype 3a. Based on the rarity of HEV RNA detection in this cohort, the investigators concluded that widespread HEV screening is not warranted. Although rare, chronic HEV infection should still be considered in immunosuppressed individuals with unexplained transaminitis.

Although practitioners must still consider HEV infection among individuals with unexplained hepatitis, data from cohort studies suggest it is a rare cause of clinical hepatitis infection among those with HIV infection in the United States.

In a study of HEV seroprevalence from Rakai, Uganda, 500 HIV-infected individuals were tested for HEV immunoglobulin G (IgG) along with 500 uninfected age- and sex-matched controls (Abstract 593). Samples that tested positive for IgG as well as all samples from those with CD4+ cell counts below 200/µL were also tested for HEV IgM. All samples that tested positive for IgM were then tested for HEV RNA along with random samples from those who tested positive for IgG or had a CD4+ cell count below 200/µL. Overall, the seroprevalence of HEV IgG was 47% with no difference between HIV-infected and uninfected individuals. HEV seroprevalence was associated with male sex, as has been consistently described. Only 1 sample (out of 480 tested) was positive for HEV IgM. This sample was also the only one positive for HEV RNA (out of 42 tested). This study supports the known endemicity of HEV infection in Africa but further suggests that HIV positivity is not a risk factor for HEV exposure.

Hepatitis Delta

Coinfection with hepatitis delta virus (HDV) and chronic HBV infection is relatively rare in the United States but is likely underdiagnosed. HBV/HDV coinfection leads to accelerated progression of liver disease, and there are no proven effective therapies for HDV infection.[27] Case reports have suggested that prolonged HBV DNA suppression with TDF-containing treatment may have some impact on HDV RNA levels and disease course.[28] In an analysis from the Swiss HIV Cohort, 139 of 771 individuals who tested positive for HBsAg also tested positive for anti-HDV antibodies. Of these, 122 had samples available for HDV polymerase chain reaction (PCR) testing, and 73 (60%) of these samples tested positive for HDV RNA. Interestingly, 20 of the 49 individuals with negative PCR test results also had negative results on repeat anti-HDV antibody testing performed using a different test, suggesting that 15% of the original positive anti-HDV results were false positives.

Overall HDV seroprevalence was 12.8%, and 74% had a detectable HDV viral load at a median of 4.7 $\log_{10}$ copies/mL. A subset of 20 individuals with detectable HDV RNA who were taking TDF-containing antiretroviral therapy were followed for a median of 34 months. Although the mean HDV RNA level fell in the group during treatment with TDF (from 8.1 $\log_{10}$ copies/mL to 7.2 $\log_{10}$ copies/mL), only 25% experienced a reduction in HDV RNA level of more than 2 $\log_{10}$ copies/mL. HDV RNA became undetectable in 3 individuals during the follow-up period. In contrast, HBV DNA was suppressed to below 200 IU/mL in all 20 individuals. This result is unsurprising given the relatively short duration of TDF exposure and the lack of a profound impact of TDF use on HBsAg decline or loss. Novel HBV antiviral drugs that better target HBsAg production or novel HDV inhibitors may offer improved therapeutic options for HDV/HBV coinfection in the future.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

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Invited Review

CROI 2016: Advances in Antiretroviral Therapy

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The 2016 Conference on Retroviruses and Opportunistic Infections highlighted exciting advances in antiretroviral therapy, including important data on investigational antiretroviral drugs and clinical trials. Clinical trials demonstrated benefits from a long-acting injectable coformulation given as maintenance therapy, examined intravenous and subcutaneous administration of a monoclonal antibody directed at the CD4 binding site of HIV-1, and provided novel data on tenofovir alafenamide. Several studies focused on the role of HIV drug resistance, including the significance of minority variants, transmitted drug resistance, use of resistance testing, and drug class–related resistance. Novel data on the HIV care continuum in low- and middle-income settings concentrated on differentiated HIV care delivery models and outcomes. Data on progress toward reaching World Health Organization 90-90-90 targets as well as outcomes related to expedited initiation of HIV treatment and adherence strategies were presented. Results from a trial in Malawi showed reduced rates of mother-to-child transmission among HIV-infected women who initiated antiretroviral therapy prior to pregnancy, and several studies highlighted the effect of antiretroviral therapy in pediatric populations. A special session was dedicated to the findings of studies of Ebola virus disease and treatment during the outbreak in West Africa.

Keywords: CROI, 2016, HIV, antiretroviral, drugs, therapy, clinical trials, long-acting injectable agent, resistance, care delivery, resource-constrained settings, Ebola

Investigational Antiretroviral Agents

MK-8591

At the 2016 Conference on Retroviruses and Opportunistic Infections (CROI), Grobler and colleagues presented data on MK-8591, an investigational nucleoside analogue reverse transcriptase inhibitor (nRTI) (4'-ethynyl-2-fluoro-2'-deoxyadenosine or EFdA) (Abstract 98). It is highly potent in vitro, with a 50% effective concentration (EC_{50}) of 0.2 nM. MK-8591 differs from other nRTIs in that it retains a 3'-hydroxyl group. This compound acts as a translocation inhibitor and, as a result, causes inefficient chain elongation. In a monkey model, the intracellular half-life of the phosphorylated metabolite of MK-8591 is approximately 50 hours, suggesting the potential for once-weekly dosing. This was confirmed in a model that used simian immunodeficiency virus (SIV)-infected rhesus macaques. In a phase I repeated-dose study, the investigators found that MK-8591 10 mg weekly resulted in trough concentrations that exceeded the target concentration by 2 fold and that the compound was well tolerated. An extended-release parenteral formulation that exhibits therapeutic levels for more than 180 days after a single injection is under investigation. In a separate investigation, a single dose of MK-8591 10 mg given to HIV-infected adults resulted in a 1.6 $\log_{10}$ copies/mL decline in plasma HIV-1 RNA through 10 days, with an intracellular half-life of 4.3 days and no emergence of resistance (Abstract 437LB).

BMS-986197

Krystal and colleagues presented preclinical data on BMS-986197, an investigational recombinant biologic molecule that incorporates 3 HIV entry inhibitors using small proteins derived from human fibronectin called adnectins (Abstract 97). The entry inhibitors include 2 anti–glycoprotein 41 adnectins binding at 2 different locations and an anti-CD4 adnectin joined into a single molecule. The combination of the individual components exhibits synergy in vitro and appears to have a heightened barrier to resistance. The pharmacokinetic data from a primate model support once-weekly subcutaneous dosing, and preliminary evidence of activity in vivo was observed in a humanized mouse model.

ABX-464

Scherrer and colleagues presented data on ABX-464, an investigational compound that inhibits HIV replication of Rev (Abstract 461LB). The investigators tested ascending doses in HIV-infected adults and found that the compound was tolerable with no concerning safety events. The compound exhibited modest antiretroviral activity, with 4 of 6 participants experiencing a 0.5 $\log_{10}$ copies/mL reduction in plasma HIV RNA after 14 days of dosing.

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BMS-955176

BMS-955176 is an investigational maturation inhibitor currently in phase II trials. Ray and colleagues presented data on the in vitro susceptibility of clinical isolates from individuals whose protease inhibitor (PI)-based therapy failed and a separate panel of highly PI-resistant viruses (Abstract 464). The investigators found that BMS-955176 retained activity against these viruses, suggesting that this compound will be useful for participants with highly drug-resistant HIV.

Clinical Trials of Long-Acting Injectable Agents

Injectable Cabotegravir and Rilpivirine for Maintenance Therapy

Margolis and colleagues presented results from the LATTE-2 (Long-Acting Antiretroviral Treatment Enabling–2) trial, a randomized open-label trial of the investigational long-acting integrase strand transfer inhibitor (InSTI) cabotegravir with rilpivirine in an injectable coformulation given as maintenance antiretroviral therapy (Abstract 31LB). Three hundred nine antiretroviral therapy–naive participants (92% male, 20% nonwhite, and 18% with a plasma HIV-1 RNA level > 100,000 copies/mL) initiated treatment with cabotegravir plus abacavir and lamivudine given orally for 20 weeks; rilpivirine was added for weeks 16 through 20. Virologic suppression was achieved in 91%, and 286 were then randomly assigned 2:2:1 to maintenance therapy with the injectable regimen given every 8 weeks or every 4 weeks or to continuation of the oral regimen. The primary efficacy end point was virologic suppression 32 weeks after randomization. Virologic suppression, according to the US Food and Drug Administration (FDA) Snapshot algorithm, was observed among 95% of participants who received the injectable regimen every 8 weeks, 94% of participants who received the injectable regimen every 4 weeks, and 91% in the oral dosing arm. Each of the arms receiving the injectable regimen achieved noninferiority. Among the participants with a plasma HIV-1 RNA level greater than 50 copies/mL at week 32, all continued on their randomized therapy and achieved virologic suppression at subsequent time points. Two participants developed protocol-defined virologic failure and none had emergence of resistance mutations.

A long-acting injectable coformulation for maintenance antiretroviral therapy is moving forward to phase III trials.

There were statistically significantly more grade 3 or 4 adverse events (not including injection site reactions) in the injectable arms, although most of these were deemed unrelated to study treatment. Injection site reactions (pain, swelling, and nodules) were common in the injectable treatment arms; nearly all reactions were grade 1 or 2, and the frequency declined over time. Only 2 participants (1%) withdrew because of injection site reactions. Participants in the injectable treatment arms reported a very high rate of satisfaction with their assigned treatment. This injectable combination is moving forward to phase III trials.

VRC01

Mayer and colleagues presented data on intravenous and subcutaneous administration of VRC01, a broadly active monoclonal antibody directed at the CD4 binding site of HIV-1, to 88 HIV-uninfected volunteers (Abstract 90). VRC01 was well tolerated, with 6.2% of participants experiencing adverse events (all mild) related to the study product. VRC01 exhibited a pharmacokinetic profile that supports subcutaneous dosing every 3 weeks or intravenous dosing every 2 months.

Bar and colleagues also presented data on VRC01 in HIV-infected adults (Abstract 32LB). The investigators enrolled participants who were virologically suppressed for at least 6 months, who had a current CD4+ cell count greater than 400/µL, and who had a CD4+ cell count nadir greater than 200/µL. VRC01 was administered intravenously every 3 weeks for 3 doses and antiretroviral therapy was interrupted 1 week after the first dose of VRC01. Participants were monitored weekly. Fourteen participants were enrolled; 1 participant was not evaluable because he discontinued antiretroviral therapy prior to the first infusion. All participants were men and 50% were white. The infusions were safe and well tolerated, and plasma VRC01 concentrations were in the anticipated range throughout the study. Eleven of 13 participants experienced virologic rebound by week 5 and the other 2 experienced it by week 12. Clonal analyses of virus isolated during virologic rebound suggest clonal expansion of virus with preexistent VRC01 resistance in some of the participants. The investigators suggested that future studies should examine combinations of broadly neutralizing antibodies.

Other Clinical Trials of Antiretroviral Therapy

Switching to Tenofovir Alafenamide

Gallant and colleagues presented results from a randomized, double-blind, double-dummy clinical trial of changing stably suppressed individuals taking emtricitabine and tenofovir disoproxil fumarate (TDF) to emtricitabine and tenofovir alafenamide (TAF) (Abstract 29). This trial randomly assigned 663 participants (15% female, 25% nonwhite, 46% receiving a boosted PI) to continue emtricitabine and TDF or start emtricitabine and TAF. Placebo tablets were used to mask treatment assignment, and the dose of emtricitabine and TAF depended on use of a boosted PI. The primary end point was virologic suppression at week 48, using the FDA snapshot algorithm: 94.3% for emtricitabine and TAF compared with 93% for emtricitabine and TDF (1.3%; 95% confidence interval [CI], -2.5%-5.1%) achieving noninferiority. The end points were driven by lack of data at week 48 not virologic failure. There were statistically significant improvements in renal tubular biomarkers and estimated glomerular filtration rates in the emtricitabine and TAF group compared with emtricitabine and TDF. There were statistically significant increases in bone

density with the use of emtricitabine and TAF compared with the stable bone density observed with emtricitabine and TDF. There were statistically significantly higher levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides with emtricitabine and TAF than with emtricitabine and TDF. These results support the safety and efficacy of switching emtricitabine and TDF to emtricitabine and TAF to avoid potential renal and bone toxic effects.

Second Antiretroviral Regimens in Resource-Constrained Settings

La Rosa and colleagues presented data from the AIDS Clinical Trials Group (ACTG) A5273 trial, a randomized clinical trial of ritonavir-boosted lopinavir plus 2 nRTIs compared with boosted lopinavir plus raltegravir for individuals whose initial nonnucleoside analogue reverse transcriptase inhibitor (NNRTI)-based regimen failed (Abstract 30). This was a phase III, open-label, randomized clinical trial conducted at 15 sites in 9 countries. Five hundred twelve participants (52% female, 64% black, 81% infected with subtype C) with a median CD4+ cell count of 135/μL and plasma HIV-1 RNA level of 4.5 $\log_{10}$ copies/mL were randomly assigned to one of the study arms. The cumulative probability of virologic failure through week 48 was 10.3% in the raltegravir-containing arm and 14% in the nRTI-containing arm. This achieved noninferiority but did not demonstrate statistical superiority of raltegravir. Participants with 3 or more nRTI resistance mutations at baseline were more likely to achieve virologic suppression in each arm. The investigators suggested that baseline nRTI resistance was a marker of adherence. These data support the current World Health Organization (WHO) recommendation for boosted lopinavir plus an nRTI as a second antiretroviral regimen.

Additional Pharmacokinetic Considerations

Colbers and colleagues evaluated the pharmacokinetics of a crushed fixed dose of elvitegravir, cobicistat, emtricitabine, and TDF (Abstract 431). They found that the crushed tablet administered via feeding tube had similar pharmacokinetics to the intact tablet. The investigators concluded that it was reasonable to crush the tablet for administration. Molto and colleagues examined the effect of hemodialysis on dolutegravir concentrations (Abstract 432). The researchers found minimal removal of dolutegravir by hemodialysis and concluded that no dose adjustments are necessary in this situation.

Resistance

Transmitted Drug Resistance and Minority Variants

A themed discussion was dedicated to next-generation sequencing for detection of transmitted or linked drug resistance. Minority-level variants are defined as variants present at less than 20% of the viral population. Since minority-level variants do not uniformly lead to virologic failure in all cases, nor does all majority-level resistance lead to virologic failure, a better understanding of the breadth and clinical significance of minority-level variants is essential. The session showcased studies aimed at unraveling the highly complex nature of in vivo variant expression.

The prevalence and significance of sexual transmission of minority HIV drug resistance mutations (DRMs) is not completely understood. Chaillon presented a next-generation sequencing analysis of 31 HIV-infected, antiretroviral therapy–naive, phylogenetically and epidemiologically linked male source and recipient partners sampled within a median of 9 days (Abstract 487). Next-generation sequencing identified a total of 139 DRMs from 22 sites (9 NNRTI, 13 nRTI) at an average frequency of 3.37% (interquartile range [IQR], 0.3%-5.1%). There was no evidence of preferential sexual transmission or selection of minority DRMs. Longitudinal samples in the recipient partner showed that minority DRMs were identified only during early infection and were lost over time, suggesting a mutation-selection balance hypothesis in which deleterious mutations are efficiently purged from the population later in infection.

Minority DRMs were identified only during early HIV infection and were lost over time, suggesting a mutation-selection balance hypothesis in which deleterious mutations are efficiently purged from the population later in infection.

InSTIs have increasingly been used as initial therapy in many settings; however, little is known about InSTI-associated transmitted drug resistance. Todesco presented analysis of 92 recently diagnosed, treatment-naive individuals with HIV infection and found InSTI-associated mutations in 7.6% (2.2%-13.0%) of all samples analyzed by Sanger sequencing and 10.9% (4.5%-17.2%) of all samples analyzed by ultradeep sequencing (Abstract 489). Although none of the classic InSTI-associated resistance mutations (positions 143, 148, and 155) were identified by either sequencing method, among men who have sex with men (MSM; n = 70), rates of InSTI-associated mutations were relatively high, with a prevalence of 10% (3.05%-17.0%) by Sanger sequencing and 14.3% (6.1%-22.5%) by ultradeep sequencing. Notably, there were 3 viruses that harbored minority variant mutations that were only detected by ultradeep sequencing (2 R263K and 1 E138K). The R263K mutation has been associated with dolutegravir failure.

In an effort to identify low-frequency transmitted drug resistance and describe the evolution of DRMs over time, Singh and colleagues applied ultradeep sequencing in a cohort of 14 acutely infected individuals with HIV subtype C virus infection (Abstract 488). Using a clinical cutoff of greater than or equal to 1% to identify low-frequency DRMs, 8 of 14 participants were found to have low-frequency DRMs associated with nRTIs, NNRTIs, PIs, or InSTIs. A subset of the samples was sequenced at additional time points to monitor for reversion,

and PI- and InSTI-associated mutations were found to revert by 7 days to 12 days; however, the NNRTI-associated K103N mutation persisted in 1 participant for nearly a year. The investigators also acknowledged that although the K65R mutation was the most common DRM, this may have been related to technical error in the context of 454 sequencing technology, which has been identified as a spurious finding in other analyses of 454 sequencing.[1]

Targeted next-generation sequencing is a powerful tool for detecting low-frequency, HIV drug–resistant mutations, but polymerase chain reaction (PCR) error and frequent in vitro recombination prevent accurate detection of linked mutations and assessment of population structure. Boltz presented promising results using a new next-generation sequencing–based ultrasensitive single-genome sequencing assay designed to minimize PCR bias, error, recombination, and resampling (Abstract 490). The ultrasensitive single-genome sequencing method utilizes primer IDs, short primers, and efficient ligation rather than primer IDs and long primers as is the case with standard methods. Using samples from a donor whose antiretroviral regimen failed, ultrasensitive single-genome sequencing was compared with standard methods and was found to accurately identify clustering and correct PCR error.

Transmitted Drug Resistance

Guidelines continue to recommend boosted PI–based antiretroviral therapy for those individuals found to have thymidine analogue mutations (TAMs) on baseline resistance testing, to improve the barrier to resistance. Geretti and colleagues analyzed Sanger sequences and clinical data from the UK CHIC (United Kingdom Collaborative HIV Cohort) study to examine virologic outcomes of initial antiretroviral therapy in HIV-seropositive adults with 1 or more TAMs as the sole form of transmitted drug resistance compared with those who did not have any detectable baseline resistance (Abstract 482). There were 269 participants with isolated baseline TAMs and 6330 participants with no evidence of baseline resistance based on the 2009 WHO resistance mutations list.[2] All participants were treated with an NNRTI-based or a boosted PI–based regimen. A multivariable model and Kaplan-Meier analyses showed that the risk of virologic failure was statistically significantly associated with use of a boosted PI–based regimen compared with an NNRTI-based regimen and that the presence of TAMs at baseline did not influence virologic responses to NNRTI-based regimens. At 5 years, the probability of virologic failure was 15.3% in the group that received an NNRTI-based regimen and 31.8% in the group that received a PI-based regimen. Notably, the most common sequencing result was a single TAM, and in a subgroup analysis, there was a trend toward higher rates of virologic failure among participants with more than 1 TAM at baseline in both treatment groups. Nonetheless, this analysis calls into question the guidance on the management of individuals with isolated TAMs at baseline and the investigators called for controlled studies to better inform recommendations.

Although transmitted drug resistance to NNRTI-based therapy is commonly reported, transmission of resistance to PIs is rare. Harrigan and colleagues described transmission and clustering of resistance to HIV PIs in Ontario, Canada (Abstract 491LB). After observing an unusual pattern of baseline resistance, investigators reviewed HIV sequences from all individuals in care and applied phylogenetic mapping for HIV *pol* sequencing from the first sample for which testing was available (N = 11,500). There were 49 treatment-naive participants with PI resistance in a single large cluster. Each of the participants had numerous PI resistance–associated mutations (typically 7) at the time of first pretreatment genotype test, which conferred substantial resistance to most PIs except darunavir. These mutations were also commonly accompanied by the nRTI-associated revertant mutations M41L and T215-L/S. Most cases were observed in late 2014 or in 2015. All 49 individuals were men (median age, 29 years), and all indicated that HIV exposure occurred through sex with men. This highly resistant cluster illustrates that substantial PI-associated resistance can occur with sufficient replicative fitness to circulate for many years and possibly threaten the current treatment paradigm. The investigators highlighted the importance of systematic surveillance of HIV resistance in untreated individuals.

A highly resistant cluster illustrated that substantial resistance to PIs can occur with sufficient replicative fitness to circulate for many years and possibly threaten the current treatment paradigm.

Transmitted Drug Resistance and Surveillance

The WHO has proposed a global standardized HIV drug resistance, monitoring, and surveillance strategy with the aim of ensuring sustainability of antiretroviral treatment programs. Avila-Rios shared results from a nationally representative pretreatment drug resistance survey in Mexico (Abstract 477). There were 264 viruses included in the study, representing a sample of 288 participants drawn from Ministry of Health clinics across the country. Pretreatment drug resistance was measured by Sanger sequencing and next-generation sequencing and based on the WHO surveillance HIV drug resistance mutation list and the Stanford calibrated population resistance tool.[2,3] Applying standard sequencing, pretreatment drug resistance to any drug was found to be 12% (95% CI, 8.4%-16.5%) and NNRTI-associated resistance was found to be the highest (6.9%), followed by nRTI-associated resistance (5.1%) and PI-associated resistance at (2.6%). The most frequent pretreatment drug resistance mutation was the K103N mutation. Additional analysis was conducted using next-generation sequencing, which showed increasing mutation frequencies as the threshold for population detection was lowered. Among individuals who had NNRTI-associated resistance at baseline, only 25% were virologically suppressed

on NNRTI-based regimens. Additionally, NNRTI-associated resistance detected in minority variants was also statistically significantly associated with failure to achieve viral suppression, which was found at a threshold as low as 5%. The investigators suggested that the integration of baseline HIV drug resistance testing could improve the efficacy of antiretroviral therapy.

Resistance Testing

Current guidelines in the United States recommend HIV resistance testing at entry into care or soon after HIV diagnosis.[4,5] There were 2 presentations from the Centers for Disease Control and Prevention (CDC) that investigated the rates of HIV resistance testing in the United States. Banez Ocfemia presented rates of genotype testing at entry into care among 1193 practitioners surveyed through the Medical Monitoring Project Provider Survey (Abstract 497). Of practitioners surveyed, 84.5% responded that they test all patients who are new to care, 8.8% responded that they test more than half (but not all), and 6.7% responded that they test half or fewer. Multivariate modeling indicated that not being an HIV specialist (compared with being an HIV specialist) and prescribing antiretroviral therapy based on CD4+ cell count (compared with prescribing antiretroviral therapy regardless of CD4+ cell count) were associated with a lower likelihood of ordering genotypic testing for all patients.

Dasgupta and colleagues analyzed data from the US National HIV Surveillance System (NHSS) for persons aged 13 years and older with HIV infection diagnosed in 2013 who were linked to care (ie, had a CD4+ cell count or a viral load test) within 3 months of diagnosis and resided in a jurisdiction with complete laboratory reporting and high reporting of nucleotide sequence data from resistance testing (Los Angeles County, California; Michigan; New York; South Carolina; Texas; and Washington). Of 9481 persons in these jurisdictions who received a diagnosis of HIV infection in 2013 and were linked to care within 3 months of diagnosis, 6181 (65%) ever received a resistance test and 4270 (69%) received a resistance test at the time of linkage to care. Substantially lower levels of drug resistance testing were observed in selected jurisdictions, among persons in areas with smaller populations (< 500,000 population size), and among men who inject drugs. The investigators highlighted the need to consider and address such differences in testing practices.

Because InSTIs are relatively new, data on InSTI resistance and resistance testing are limited. Since 2005, laboratories conducting any HIV resistance testing for residents of New York State are required to report HIV drug resistance results to the state Department of Health. Wang, on behalf of the New York State AIDS Institute, reported on rates of InSTI resistance testing and described resistance findings based on reported resistance tests between December 2009 and July 2015 (Abstract 501). There were 5627 InSTI resistance tests that were linked to the New York State HIV registry, of which 4208 (75%) had paired protease and reverse transcriptase resistance tests, and 3533 (63%) cases were stage 3 HIV infection at time of testing. Among the 4626 cases in which an InSTI resistance test was not initially performed, 63% of first InSTI resistance tests occurred 11 years or more after HIV diagnosis. Of 3515 cases of newly diagnosed HIV infection in New York State in 2014, 16% had an initial InSTI test, compared with 55% who were initially tested for protease and reverse transcriptase resistance. Multivariable logistic regression showed that the odds of having had an initial InSTI resistance test were related to race and ethnicity, transmission risk group, and region of residence. Accordingly, there were higher rates of initial InSTI resistance testing among men (18%), those of white and Hispanic race (19.1% and 19.0%, respectively), those at risk through sexual contact with MSM (21%) or sexual contact with MSM and injection drug use (22%), and residents of New York City (17.9%). Less than 1% (7/1001) of initial and 8.6% (400/4626) of noninitial InSTI resistance tests showed resistance to at least 1 of 3 InSTIs. The time from HIV diagnosis to InSTI resistance test and the proportion of individuals with advanced HIV infection suggest that, during this period, the majority of tests were ordered for long-standing cases at advanced stages of disease.

Resistance to InSTIs

There has been increasing use of InSTIs in clinical practice for treatment-naive and -experienced individuals. Lepik and colleagues performed a retrospective review of individuals cared for at the British Columbia Centre for Excellence in HIV/AIDS Drug Treatment Program from 2009 to 2015, to assess the prevalence and incidence of InSTI-associated drug resistance (Abstract 492LB). In British Columbia, Canada, the use of the InSTIs raltegravir, elvitegravir, and dolutegravir in antiretroviral regimens increased from 10% in 2009 (540 persons treated with raltegravir) to 32% in 2015 (978 persons treated with raltegravir, 500 persons treated with elvitegravir, and 1011 persons treated with dolutegravir). Among adults who had received antiretroviral therapy and had drug resistance testing performed, there were 57 individuals with intermediate- or high-level InSTI-associated resistance. The prevalence of InSTI-associated resistance per 1000 persons who were treated with antiretroviral therapy increased from 1.07 in 2009 to 6.8 in 2015 ($P < 001$, for trend; R^2, 0.99). During this period, resistance to antiretroviral drugs declined from 331 per 1000 persons treated to 285 per 1000 persons treated ($P < .001$, for trend; R^2, 0.98). Prior to 2014, all observed mutations were limited to raltegravir-associated resistance; however, in 2014 and 2015, resistance to dolutegravir and elvitegravir was observed as well. Three cases of InSTI-associated resistance emerged during therapy with dolutegravir, including 2 treatment-experienced individuals who developed the R263K mutation and 1 treatment-naive individual who was treated with an initial regimen of dolutegravir, abacavir, and lamivudine who developed the T66I mutation. The investigators pointed out that although InSTI-associated resistance remains low, it is increasing, and emerging InSTI-associated resistance is observed in treatment-naive and -experienced individuals.

There are now several FDA-approved coformulated InSTI-based regimens, including the recently approved coformulation of elvitegravir, cobicistat, emtricitabine, and TAF, which is associated with fewer renal and bone effects than have been observed with TDF.[6] Abram and colleagues presented a pooled week-48 resistance analysis of elvitegravir, cobicistat, emtricitabine, and TAF from 7 phase III clinical trials (Abstract 496). A total of 2308 participants were included in the analysis. The 7 ongoing trials included studies of treatment with elvitegravir, cobicistat, emtricitabine, and TAF in several populations: treatment-naive adults (2 trials), treatment-naive adolescents, virally suppressed individuals and virally suppressed individuals with resistance to 2 or more antiretroviral drug classes (2 switch studies), individuals with renal impairment, and individuals with HIV/hepatitis B virus (HBV) coinfection. As part of the resistance end point, participants underwent genotypic and phenotypic analysis at baseline and in the event of virologic failure or treatment discontinuation (HIV RNA level ≥400 copies/mL). Among 16 of 866 treatment-naive adults who had genotypic and phenotypic analysis, nRTI resistance–associated mutations (M184V/I, n = 7; K65R, n = 1) and primary InSTI-associated resistance mutations (T66I/A, n = 2; E92Q, n = 2; Q148R, n = 1; N155H, n = 1) emerged in 7 (0.8%). Among 2 of 50 treatment-naive adolescents who had genotypic and phenotypic testing, however, no resistance was found. Among virologically suppressed individuals, 4 of 959 had genotypic and phenotypic testing; resistance emerged in 1 individual (M184M/I), and HIV RNA was resuppressed to below 50 copies/mL before treatment discontinuation. Among 110 virologically suppressed participants with prior resistance to 2 or more antiretroviral drug classes, none met the genotypic and phenotypic analysis criteria. Among 2 of 248 renally impaired individuals who were analyzed, both had multiclass resistance detected (1 preexisting and 1 due to possible reinfection followed by resuppression of HIV RNA to < 50 copies/mL). Among 75 HBV-coinfected participants, none met the analysis criteria. The investigators pointed out that resistance at 48 weeks to 1 or more components of elvitegravir, cobicistat, emtricitabine, and TAF was rare in all studied populations, including highly treatment-experienced individuals switching to this regimen.

The InSTI dolutegravir has a high genetic barrier to resistance and has been selected in treatment-experienced individuals and in cell culture. The novel R263K substitution in integrase is a mechanism of dolutegravir resistance in InSTI treatment–naive individuals. In InSTI treatment–experienced individuals, however, resistance emerges through the accumulation of resistance substitutions for other drugs in the InSTI class. For instance, E157Q can be selected after treatment with raltegravir and can be a polymorphism present in the circulating virus as well. Anstett and colleagues investigated the effects of E157Q substitution on the emergence of R263K and its effects on enzyme biochemical function, viral infectivity, and drug resistance (Abstract 507). The E157Q and R263K substitutions were introduced into the pET15b integrase protein expression vector followed by measurement of strand transfer and DNA binding activities as well as viral infectivity and drug resistance, which were measured through the infection of TZM-bl cells and observation of luciferase production. The E157Q substitution restored impaired replication conferred by the dolutegravir resistance–associated mutation R263K, and enhanced resistance to this compound by 20 fold compared with wild-type. The investigators cautioned that because position 157 in the integrase is polymorphic, it could be present at the initiation of dolutegravir-containing therapy, which could result in a replication competent, dolutegravir-resistant virus.

NNRTIs and Barriers to Resistance

The investigational NNRTI doravirine is currently in phase III clinical trials. Lai and colleagues assessed the inhibitory quotient and the 50% inhibitory concentration (IC_{50}) of doravirine compared with rilpivirine and efavirenz in the presence of K103N, Y181C, and K103N/Y181C mutants in vitro (Abstract 506). Antiviral assays were performed using laboratory HIV-1 isolates (wild-type and mutant) and MT4-GFP cells, followed by cell infection. The infected cells were added to plates containing different NNRTIs at various concentrations. IC_{50} was calculated for each, and doravirine was found to display inhibitory quotients of 39, 26, and 21 against the K103N, Y181C, and K103N/Y181C mutants, respectively, compared with inhibitory quotients of 4.6, 1.4, and 0.8 for rilpivirine and 2.5, 60, and 1.9 for efavirenz. The investigators concluded that doravirine may have a higher barrier to resistance than rilpivirine or efavirenz.

Drug Resistance in Low- and Middle-Income Countries

HIV-1 drug resistance is an important cause of failure of second antiretroviral regimens in low- and middle-income countries. On behalf of the ACTG A5288 MULTI-OCTAVE (Management Using Latest Technologies to Optimize Combination Therapy After Viral Failure) Study, Wallis and colleagues shared an analysis of resistance testing from 665 cohort participants with viral failure taking a PI-based second regimen after prior exposure to nRTIs, NNRTIs, and PIs (Abstract 493LB). Participants were from 20 sites in 10 countries and underwent resistance testing and phylogenetic analysis. All participants had been exposed to nRTIs, most commonly lamivudine or emtricitabine (100%), tenofovir (84%), and zidovudine (76%). Almost all participants (99%) had prior exposure to NNRTIs, most commonly nevirapine (63%) followed by efavirenz (56%). At time of screening, tenofovir (67%) and lamivudine (90%) were the most commonly prescribed nRTIs with either ritonavir-boosted lopinavir (55%) or boosted atazanavir (43%). Very few participants had exposure to raltegravir (6%). At least 1 resistance mutation was detected in 96% (638/665) of participants. High- or intermediate-level resistance was common, and 519 participants (78%) had resistance to 1 or more drugs. Resistance to a single drug class was found in 21%, to 2 drug classes in 31%, and to all 3 drug classes (nRTI, NNRTI, and PI) in 26%. Of the 665 sequences, 461 (69%) showed susceptibility to the second

regimens (nRTI plus boosted atazanavir or lopinavir), and the majority was susceptible or had only low-level resistance to boosted darunavir (97%) and etravirine (79%). The investigators pointed out that clinical parameters were not predictive of the extent of resistance and called for objective measures of adherence and access to both resistance testing and new antiretroviral drugs to meet the needs of low- and middle-income countries.

Boender and colleagues also called for the availability of additional antiretroviral drugs to address PI-associated resistance during failure of a second HIV regimen in sub-Saharan Africa (Abstract 498). The PASER-M (PanAfrican Studies to Evaluate Resistance Monitoring) cohort enrolled participants at the time of switch to a second antiretroviral regimen and followed up the individuals over time, monitoring viral suppression and providing genotypic analysis when HIV RNA level was at or above 1000 copies/mL. The analysis revealed that although the majority of participants were able to achieve viral suppression with a second, PI-based regimen (85%) after up to 36 months of follow-up, nRTI-associated resistance was detected in 56% and major PI-associated resistance was detected in 21.9% of those whose second antiretroviral regimen failed, conveying reduced susceptibility to all available PIs. Additional drugs, including boosted darunavir, second-generation NNRTIs, and InSTIs, are needed to meet the needs of individuals who experience failure of their second antiretroviral regimen.

In many low- and middle-income countries, routine viral load testing is not yet available to guide the decision to switch from an initial to a second antiretroviral regimen. Instead, immunologic criteria are applied to estimate treatment failure. Ndembi and colleagues evaluated the accuracy of using immunologic criteria to predict treatment failure in a retrospective cohort study of individuals cared for from 2005 to 2014 at a teaching hospital in Nigeria (Abstract 502). Immunologic failure was defined as having a decrease in CD4+ cell count to pretherapy baseline level or a persistent CD4+ cell count of less than 100/μL after 6 months of therapy. A subset of individuals had viral load monitoring, and those who were found to have an HIV RNA level above 1000 copies/mL on 2 consecutive measurements underwent genotypic testing as well. Among individuals with immunologic failure who had viral load measurements, 43.9% had detectable viral loads. Among those with an HIV RNA level of 1000 copies/mL or higher, 21% did not have any drug resistance mutations. In contrast, individuals with virologic and immunologic failures showed accumulation of extensive drug resistance mutations. The use of immunologic criteria to drive the decision to switch antiretroviral therapy resulted in unnecessary switches for individuals who did not have genotypic evidence of resistance and potentially underestimated the extent of resistance as well. The investigators suggested that viral load and genotypic testing would be beneficial to guide decisions regarding switching of antiretroviral therapy.

The use of immunologic criteria to drive the decision to switch antiretroviral therapy resulted in unnecessary switches for individuals who did not have genotypic evidence of resistance and potentially underestimated the extent of resistance.

Although baseline resistance testing is recommended in resource-rich countries, because of cost and technical demands, it is not used routinely in low- and middle-income countries. Chung and colleagues presented data from a prospective randomized trial in Kenya of a simple oligonucleotide ligation assay (OLA) to detect key *pol* reverse transcriptase–associated mutations at codons 103, 181, 184, and 190 (Abstract 494LB). A total of 991 participants were enrolled in the study in 2013 and 2014 and were randomly assigned to receive pre–antiretroviral therapy OLA testing or standard of care. Those who had 1 or more baseline resistance mutations in the OLA arm were treated with ritonavir-boosted lopinavir rather than the standard NNRTI-based initial therapy. The overall prevalence of resistance was 8.3%. Although OLA testing was found to be feasible in Kenya, OLA testing and change to a boosted lopinavir–based regimen did not substantially impact the overall rate of virologic suppression. Rate of virologic failure was similar among those who received OLA testing (7.9%) and standard of care (7.5%). Considered from an alternative perspective, among individuals with resistance, the OLA strategy reduced virologic failure (13.9% vs 46.2%; $P < .005$). OLA testing was feasible in this clinical context, and although it did not impact overall viral suppression in the as-treated analysis, it did reduce virologic failure in individuals found to have resistance. OLA testing could be a useful strategy in the event that transmitted drug resistance continues to rise.

Resistance and Implications for Preexposure Prophylaxis

Antiretroviral therapy is used for treatment of HIV infection and, increasingly, for preexposure prophylaxis (PrEP) against HIV infection. TDF is a key component of HIV treatment and PrEP approaches, but little is known about the regional burden of TDF-associated resistance and the risk factors for its emergence. Gupta presented findings from an international multicenter retrospective study of individuals undergoing genotypic testing following virologic failure with initial TDF-containing antiretroviral therapy with lamivudine or emtricitabine plus either efavirenz or nevirapine (Abstract 503). Tenofovir-associated resistance was defined as the presence of the K65R/N or K70E/G/Q mutation at treatment failure. The prevalence of TDF-associated resistance among 1926 individuals with treatment failure in 36 countries was highest in low- and middle-income countries (59.8% in West and Central Africa, 55.9% in East Africa, 55.2% in Southern Africa, 39% in Asia, and 35.3% in Latin America) and lowest in high-income regions (18.8% in Western Europe and 22.6% in North America). A multiple logistic regression analysis revealed substantial independent risk factors for TDF-associated resistance across regions: a preantiretroviral therapy CD4+

cell count below 100/μL (odds ratio [OR], 1.49 [1.26-1.77]), use of lamivudine compared with emtricitabine (OR, 1.49 [1.20-1.84]), and use of efavirenz compared with nevirapine (OR, 1.46 [1.28-1.67]). The investigators concluded that TDF-associated resistance emerges in a high proportion of individuals who develop virologic failure during treatment with a TDF-containing initial antiretroviral regimen in low- and middle-income regions and urged minimization of viral failure, early detection of viral failure, and proactive switching of therapy.

Improving HIV Care Delivery and Outcomes in Low- and Middle-Income Countries

New Data on Treatment of Children in Low- and Middle-Income Countries

Two important new investigations shed light on treatment strategies for HIV-infected children in low- and middle-income countries. Njuguna and colleagues (Abstract 38) presented results from the PUSH (Pediatric Urgent Start of Highly Active Antiretroviral Treatment) trial, in which they examined initiation of antiretroviral therapy for hospitalized HIV-infected children (birth to age 12 years) on an urgent (within 48 hours of enrollment) versus poststabilization (within 7-14 days of enrollment) basis at 4 hospitals in Kenya. The researchers screened 250 children and randomly assigned 183 to 1 of the 2 arms, but a data and safety monitoring board stopped the study at their second assessment because of futility. Substantial mortality (21%) was observed at 6 months, an outcome that did not differ between study arms (hazard ratio [HR], 1.26; 95% CI, 0.67, 2.37; P = .47). There was also no statistically significant difference in incidence of immune reconstitution inflammatory syndrome (IRIS) or serious adverse events between study arms. The investigators noted that there was a high mortality rate among these children despite the fact that all of them initiated antiretroviral therapy within 2 weeks of enrollment and that timing within those 2 weeks did not make a difference, highlighting the need for diagnosis and initiation of antiretroviral treatment prior to hospitalization for children with symptomatic disease. Early initiation of antiretroviral therapy was feasible and was not associated with adverse events such as IRIS; therefore, despite the lack of impact of urgent initiation, the results of the study were supportive of rapid initiation of antiretroviral therapy for children hospitalized with HIV infection who have comorbid illness.

Murnane and colleagues presented 4-year outcomes from the NEVEREST III (Treatment Options for Protease Inhibitor-Exposed Children) study, a randomized open-label noninferiority trial of 298 children younger than 3 years in Johannesburg, South Africa, exposed to nevirapine for prevention of mother-to-child transmission (PMTCT) (Abstract 39). The children were initially treated and achieved virologic suppression with a boosted lopinavir–based regimen, then randomly assigned to continued treatment with the boosted lopinavir–based regimen or to treatment with an efavirenz-based regimen.

One-year results of the NEVEREST III trial were published and showed that efavirenz was not inferior to boosted lopinavir in children previously exposed to nevirapine for PMTCT.[7] These results, presented as 3-year outcomes in the abstract and expanded to 4-year outcomes for the oral presentation, are from an intention-to-treat analysis of a long-term observational follow-up study of 80% of the participants from the randomized control trial. At 48 months postrandomization, 22% of the children randomly assigned to the arm containing boosted lopinavir had switched to efavirenz, and 5% of the children randomly assigned to the arm containing efavirenz had switched to boosted lopinavir. There was no statistically significant difference in time to virologic failure, defined as 2 HIV RNA level measurements above 1000 copies/mL, between those who received ritonavir-boosted lopinavir (12% failure rate) and those who received efavirenz (7% failure rate; P = .21).

In secondary analyses, efavirenz did reduce the risk of having any HIV RNA level measurement above 1000 copies/mL (OR, 0.52; 95% CI, 0.28, 0.98; P = .04) and of having HIV RNA levels between 51 copies/mL and 1000 copies/mL (OR, 0.67; 95% CI, 0.51, 0.88; P = .004) over 4 years compared with boosted lopinavir. There were also statistically significantly higher CD4+ cell counts and lower risks of abnormal cholesterol and triglyceride levels among children receiving efavirenz-based regimens. The investigators concluded that switching young children from a ritonavir-boosted lopinavir–based regimen to an efavirenz-based regimen, despite nevirapine exposure for PMTCT, is feasible and has the advantages of improved palatability, once-daily dosing, lower cost, fewer interactions with antituberculosis drugs, and preserving subsequent treatment options without conferring the disadvantage of more frequent virologic failure. These findings have important implications for treatment strategies for children in low- and middle-income countries.

Switching from a ritonavir-boosted lopinavir–based regimen to an efavirenz-based regimen, despite nevirapine exposure for PMTCT, is feasible and has many advantages for young children.

New Findings Regarding the HIV Care Continuum in Low- and Middle-Income Countries

Considering the impact of various PMTCT care models on the HIV care continuum, Abrams and colleagues presented the results of the Safe Generations study (Abstract 34). This stepped-wedge trial examined the impact of transition from the PMTCT strategy Option A, in which only those pregnant women with CD4+ cell counts at or above 350/μL are eligible for lifelong antiretroviral therapy, to Option B+, in which all pregnant women are offered lifelong antiretroviral therapy. The investigators collected data from 12 facilities, 10 of which transitioned from Option A to Option B+ (1 each month over a 14-month period) and 2 of which did not (serving as

controls). Analysis was conducted using monthly facility-level cohorts. Pregnant women not taking antiretroviral therapy were enrolled as they attended their first antenatal clinic visit. As anticipated, uptake of antiretroviral therapy was significantly greater with Option B+ (94% of 1043 women) than with Option A (35% of 1272 women; $P < .0001$). Antenatal retention, defined as clinic attendance within 56 days prior to delivery, was 54% in the Option A arm and 68% in the Option B+ arm (adjusted relative risk [aRR], 1.23; 95% CI, 1.09, 1.37; $P < .001$), after adjustment for age, CD4+ cell count, gestation at first antenatal clinic visit, and known HIV serostatus. There were statistically significant variations in retention among those taking antiretroviral therapy, with improved retention in those few with CD4+ cell counts above 350/µL in Option A cohorts. Postnatal retention at 6 months after delivery was poor (37% overall) but higher in Option B+ than Option A cohorts (50% vs 26%; aRR, 1.56; 95% CI, 1.15, 1.32). The take-home message from this implementation science approach is that although Option B+ substantially increased initiation and coverage of antiretroviral therapy for pregnant women, overall retention in postnatal care was poor. It is also concerning that proportionally more women initiating antiretroviral therapy were retained in care under Option A, implying that there could be a cost to retention as more women initiate therapy under Option B+.

Although Option B+ substantially increased initiation and coverage of antiretroviral therapy among pregnant women, overall retention in postnatal care was poor.

Mugglin and colleagues used prospective data from 20 clinics in Malawi to examine the impact of the impressive national scale-up of antiretroviral therapy, from 3000 to more than 500,000 individuals from 2004 to 2014, on retention in care by age (Abstract 118). Discontinuation rates, defined as no recorded clinic visits or pharmacy refills for more than 150 days after first missed appointment and not allowing for reentry into care, were 202 per 1000 person-years of follow-up to 5234 per 1000 person-years of follow-up in the first year of therapy and lower thereafter (35/1000 person-years of follow-up to 475/1000 person-years of follow-up). Discontinuation rates improved over time, and the investigators speculated that this had to do with more tolerable antiretroviral regimens becoming available. Discontinuation rates were statistically significantly higher for children aged 0 years to 3 years and adolescents aged 15 years to 24 years, although the discontinuation rate for adolescents decreased over time. Discontinuation was also statistically more likely for women receiving antiretroviral therapy as a component of Option B+ for PMTCT than for women receiving it based on their CD4+ cell count. These data highlight the need to focus on care engagement for adolescents and pregnant women, particularly in the first year of antiretroviral therapy.

Tiendrebeogo and colleagues examined retention in care after 6 years of antiretroviral therapy and CD4+ cell count response to antiretroviral therapy by sex in an International Epidemiological Databases to Evaluate AIDS (IeDEA) cohort in West Africa (Abstract 1015). The cohort included 49,677 people living with HIV infection who initiated antiretroviral therapy, 66% of whom were women, across 9 West African countries. The probability of retention failure (ie, death or loss to follow-up) at 6 years was 50% for men and 43% for women, a statistically significant difference. This difference was more dramatic after 6 months of antiretroviral therapy. Consistent with this, women had statistically significantly greater increases in CD4+ cell count after initiation of antiretroviral therapy, with women gaining an additional 1.15/µL per month (95% CI, 1.02, 1.29) compared with men. The mean CD4+ cell count for women after 6 years of antiretroviral therapy was more than 500/µL, whereas for men it was approximately 400/µL, also a statistically significant difference.

In an overview of issues of equity between sexes throughout the HIV care continuum, Ayles (Abstract 120) discussed findings from the PopART (Population Effects of Antiretroviral Therapy to Reduce HIV Transmission) study, including that once diagnosed with HIV infection, men were equally as likely as women to be referred to HIV care and to initiate antiretroviral therapy, and other data supporting the conclusion that men are more likely than women to be lost to follow-up.[8] Ayles proposed a model of clinics tailored to address these disparities, as has been seen in Western Cape, South Africa, where there are now 8 HIV treatment centers catering specifically to men.

To assess the impact of population mobility on the success of test-and-treat initiatives to achieve population-level virologic control, Larmarange and colleagues used data from the French Agence Nationale de Recherche sur le Sida (ANRS) 12249 Antiretroviral HIV Treatment as Prevention trial, a cluster randomized proof-of-concept trial in a rural area of northern KwaZulu-Natal, South Africa (Abstract 169LB). The investigators examined data from the first 4425 individuals included in the trial, 22% of whom were living with HIV infection. To assess the impact of population mobility on the impact of treatment as prevention, residency status (defined as spending at least 4 nights per week in the city area) was computed for each individual by day using data on trial registration, migration, and death. Because this trial utilized repeated cross-sectional approaches, it can demonstrate a dynamic cascade per calendar time, which is more robust than single cross-sectional approaches that do not account for population mobility. The investigators found high levels of migration in and out throughout the study period. If a strict calendar approach was used, the prevalence of virologic suppression increased from 25% to 40% over 15 months. Only 15% of HIV-infected people entering the population were virologically suppressed, and 27% of those migrating out were virologically suppressed. However, when individual-level data presented by exposure time were used, the percentage of virologically suppressed individuals increased from approximately 20% to 50% over 30 months, demonstrating that population mobility attenuates the observed impact of

treatment-as-prevention strategies when single measurements of cross-sectional data are used. The investigators also suggested that universal test-and-treat strategies incorporate interventions to ensure continuity of care for migrants in order to maximize impact.

Progress in Reaching 90-90-90

Since the Joint United Nations Programme on HIV/AIDS (UNAIDS) announced its ambitious 90-90-90 treatment goals (that 90% of all HIV-infected individuals will be aware of their HIV serostatus, 90% of those who are aware of their serostatus will be receiving antiretroviral therapy, and 90% of those receiving antiretroviral therapy will be virally suppressed by 2020) in 2014,[9] much of the discussion regarding the HIV care continuum in low- and middle-income countries has focused on whether national treatment programs will be able to achieve these targets. Many investigators presented exciting data on progress toward these goals, and the achievements of programs in Botswana and Malawi were impressive examples of the feasibility of reaching 90-90-90.

The achievements of Option B+ programs in Botswana and Malawi were impressive examples of the feasibility of reaching 90-90-90.

Gaolathe and colleagues presented preliminary data from the Botswana Combination Prevention Project trial, a pair-matched community randomized trial underway in 30 communities in Botswana, which will offer annual surveys of incidence and uptake of HIV prevention interventions (Abstract 111). Data from a preintervention survey of 12,610 individuals (81% of eligible participants) from the 30 communities were collected; HIV testing was offered to all residents without written documentation of prior HIV infection status, and HIV RNA testing was conducted for all HIV-infected participants. Overall, 29% of participants were living with HIV infection, 83% (95% CI, 81, 85%) of whom were already aware of their serostatus and could provide documentation. Including participants who reported HIV infection without documentation, 87% were aware of their HIV serostatus. Of those who knew their HIV serostatus, 87% (95% CI, 86, 89%) were receiving antiretroviral therapy; of those, 96% had an HIV RNA level of 400 copies/mL or lower, the national criterion for virologic suppression. Based on these data, rate of virologic suppression among all those living with HIV infection was 70%, only 3 percentage points under the 90-90-90 goal of 73%. These high levels of coverage throughout the care cascade are particularly interesting because current national guidelines only provide free antiretroviral treatment to citizens with CD4+ cell counts at or below 350/μL. These data suggest that the goal of 90-90-90 is ambitious but achievable and are highly encouraging for other low- and middle-income countries.

Jahn presented data from 717 sites within the Malawi national PMTCT program, to estimate the impact of Option B+ on national levels of antiretroviral treatment coverage (Abstract 168LB). Prior to the roll out of Option B+ in 2011, approximately 49% of pregnant women were aware of their HIV serostatus, 3% were receiving antiretroviral therapy, and 2% were virologically suppressed. In 2015, after implementation of Option B+, 80% of pregnant women were aware of their HIV serostatus, 78% were receiving antiretroviral therapy, and 48% were virologically suppressed. Based on changes over the period from 2005 to 2015, the investigators predicted that women will meet the 90-90-90 goal of 81% receiving antiretroviral treatment by 2017 and that average antiretroviral treatment coverage for men and women will reach 81% by 2020. Even with these impressive gains, the goal of 90% of pregnant women being aware of their HIV serostatus has not been met, and efforts to improve perinatal counseling and HIV testing are needed.

Jahn also discussed the scale up of Option B+ in Malawi in the context of the larger national response to the HIV epidemic (Abstract 119). The success of the program is evident, with dramatic increases in the proportion of women already taking antiretroviral therapy during pregnancy: from 26% in 2012 to 53% in 2015. However, challenges to retention in care remain, with a substantial decline in engagement in care in the first 6 months of antiretroviral therapy for those treated under Option B+ and no notable improvement in rates of retention in care from 2010 to 2015. There was also a wide range of rates of retention in care by site (50%-100%), emphasizing the need for continued monitoring and evaluation as Malawi begins a universal test-and-treat strategy in 2016.

Fidler and colleagues discussed findings on population-level antiretroviral treatment coverage using data from the first round of intervention in the PopART study (Abstract 114).[10] In the PopART study, annual rounds of home-based voluntary HIV testing by community HIV care practitioners were followed by health promotion; active referral and support for retention in care by the community practitioners; and voluntary male medical circumcision, PMTCT, HIV treatment, promotion of sexual health, tuberculosis treatment, and condom provision for appropriate populations.[10] Fidler presented data on the cascade of engagement in care within the intervention arm only. Of 4139 HIV-infected men, 47% were already engaged in care and taking antiretroviral treatment. Of those referred to care, 44% had initiated treatment within 6 months, and 60% had initiated treatment within 12 months. Of 8701 women, 49% were already receiving treatment. Of those newly referred to care, 42% initiated treatment within 6 months, and 56% initiated treatment within 12 months. Of participants who were offered immediate treatment in the clinic with CD4+ cell counts of or below 500/μL, 99% accepted it. At the end of the first year of intervention, the percentage of HIV-infected individuals not taking antiretroviral treatment declined from 51% to 29%, a substantial step toward the 90-90-90 goal. However, time to initiation of treatment was still slower than wished, with only 60% of those who were eligible receiving antiretroviral treatment after 12 months of intervention.

Chiu and colleagues used an integrated set of 3 mathematical models, 2 epidemiologic and 1 cost, to identify the

most cost-effective combination of 27 potential interventions in South Africa under the current budget or with the goal of reaching the 90-90-90 targets (Abstract 115). Considering the high impact and coincidence of the tuberculosis and HIV epidemics in South Africa, the investigators incorporated 2 different tuberculosis-related scenarios: the first was the current baseline in South Africa and the second was dictated by the 90-90-90 targets for tuberculosis. Under the current budget, condom availability and male medical circumcision were cost saving, and antiretroviral therapy at current national guidelines and PMTCT both had acceptable incremental cost-effectiveness ratios of $106 and $138, respectively, per life-year saved. The cost of universal antiretroviral treatment was $243 per life-year saved, and the 90-90-90 targets for HIV care were reached only when high coverage of adherence clubs—antiretroviral treatment delivery and adherence support groups run by nonmedical personnel—was added to the model. The investigators also found that the 90-90-90 targets for tuberculosis could not be met solely by optimizing care along the HIV care continuum and required optimization of tuberculosis care as well. These data emphasize the need for a multipronged approach to the HIV and tuberculosis epidemics in South Africa and the difficult financial outlay that is still needed before these epidemics are contained and costs begin to fall.

Improved Treatment Outcomes and Assessment of Care Quality for HIV-Infected Adults in Low- and Middle-Income Countries

Mortality Within HIV Treatment Programs. CROI 2016 included data from many different settings on mortality and outcomes at various points in the HIV care continuum in low- and middle-income countries. Mooser and colleagues conducted a systematic review of 34 studies in 26 cohorts that examined outcomes for adults and children lost to follow-up in sub-Saharan Africa (Abstract 1021). Mortality in those lost to follow-up declined from 53.1% in 2003 to 19.8% in 2010 ($P = .003$), and the proportion of individuals transferred to different care sites remained stable at 26%. Notably, 31.5% of individuals were traced but not found. An increase in median CD4+ cell count from 71/μL to 138/μL at initiation of antiretroviral therapy was seen over the same time period in the 9 studies reporting these data, and this increase could be responsible for improvements in mortality rates among those lost to follow-up. The review was limited by varying definitions of loss to follow-up (ranging from several weeks to 6 months) and differences in tracing methods, but the findings are encouraging.

Bendavid and colleagues examined mortality associated with failure to progress along various stages of the HIV care cascade (Abstract 117). The researchers examined data from the ALPHA (Analyzing Longitudinal Population-based HIV/AIDS data on Africa) network, in which investigators estimated that 40% of excess deaths among HIV-infected persons occurred among those who were diagnosed but not yet in HIV care, and estimates from Western Cape, South Africa, where linkage between a death registry and clinical records was used to determine that 25% of deaths occurred among undiagnosed HIV-infected persons and that 35% of deaths occurred among those who were diagnosed but not linked to care. The researchers compared these estimates to those from individual-level mathematical models of the HIV epidemics in Rwanda, Kenya, Malawi, and South Africa, in which an estimated 25% to 40% of deaths among persons living with HIV infection were among those who had never initiated antiretroviral treatment, similar to estimates from empiric datasets. The models were then used to estimate the impact of immediate initiation of antiretroviral therapy, which led to a 6% to 14% reduction in deaths over the next 10 years, mostly derived from prevention of loss to care rather than clinical benefits or reductions in HIV transmission. These data highlight the need to streamline initiation of antiretroviral therapy if mortality rates among persons living with HIV infection are to approach those in uninfected populations.

Initiation of antiretroviral therapy must be streamlined if mortality rates among persons living with HIV infection are to approach those in uninfected populations.

Dolling and colleagues presented a retrospective analysis of the relationship between mortality after 48 weeks of antiretroviral therapy and virologic suppression from the DART (Development of Antiretroviral Therapy in Africa) study, a randomized controlled trial in Uganda and Zimbabwe that compared monitoring of CD4+ cell count and other laboratory studies every 12 weeks with clinically driven laboratory monitoring (Abstract 1027).[11] During the course of the original study, no HIV RNA testing was available in real time. For this analysis, the investigators examined HIV RNA measurements taken 6 weeks to 13 weeks prior to death among those who died after 48 weeks of antiretroviral therapy; 41% of these deaths occurred in the setting of virologic suppression (HIV RNA level ≤200 copies/mL). Death in the setting of virologic suppression was more likely to be caused by gastrointestinal illness (12% in those who were virologically suppressed vs 2% in those with an HIV RNA level >200 copies/mL; $P = .04$) or HIV-related malignancies (12% in those who were virologically suppressed vs 2% in those who were not; $P = .04$). Although individuals who died with virologic suppression had higher CD4+ cell counts (238/μL) than those who died without virologic suppression (62/μL), the 2 groups did not have a statistically significant difference in the change in CD4+ cell count over the 48 weeks prior to death. These data are concerning because, despite the achievement of virologic suppression, death from non–HIV-related causes led to substantial mortality in this cohort.

Bassett and colleagues presented a unique strategy for prediction of 1-year mortality in people newly diagnosed with HIV infection by examining the impact of self-perceived barriers to health care at the time of diagnosis (Abstract 1016). The investigators enrolled 4903 individuals newly diagnosed

with HIV infection and collected responses to a 15- to 20-minute survey on demographics, emotional health, social support, and self-perceived barriers to entering HIV care, including service delivery, financial considerations, personal health perceptions, and logistical and structural barriers to care. Participants who noted barriers to entering care were more likely to die by 12 months of follow-up, even after adjustment for known predictors of mortality such as low CD4+ cell count, age, sex, and tuberculosis coinfection (for 1-3 barriers, adjusted HR [aHR], 1.68; 95% CI, 1.20, 2.34) (for >3 barriers, aHR, 2.54; 95% CI, 1.92, 3.37). No individual barrier was more predictive than the others, and social support and mental health survey data did not impact this prediction model. The investigators concluded that screening for perceived barriers to HIV care at diagnosis could identify those at high risk for mortality and perhaps provide insights into interventions for these individuals.

Measurement and Consequences of Adherence. Two groups of investigators presented novel data on adherence to antiretroviral therapy. Orrell and colleagues compared 6 adherence measurement techniques in 230 antiretroviral therapy–naive individuals in South Africa to determine which best predicts outcomes: electronic adherence monitoring devices (eg, a "smart" pill box), clinic-based 60-day pill counts, 3-day self-reports of adherence, average medication possession ratio (number of tablets/days in care) or gaps in medication (number of days without medication) based on pharmacy data, or a mid-dose assessment of efavirenz concentration (Abstract 1029). Electronic adherence monitoring and pharmacy refill data (average possession ratio and gaps) were most predictive of virologic failure, defined as an HIV RNA level above 40 copies/mL at week 48 of antiretroviral therapy, and the emergence of drug resistance, determined by detection of any resistance mutation (using the 2015 drug resistance mutations in HIV-1 from the IAS–USA[12]) on an HIV genotype test at week 48. Of concern, a 3-day recall self-report of adherence was not predictive of virologic failure or the emergence of resistance; pill counts were only modestly predictive, and efavirenz concentrations were predictive of resistance but not virologic failure. The investigators concluded that pharmacy refill data is an underutilized but often widely available methodology for clinically relevant and actionable measurement of adherence.

Collier and colleagues presented the results of a retrospective analysis of 307 adults who initiated a second antiretroviral regimen that included a PI and who resided within the Africa Centre's demographic surveillance area, a largely rural and socioeconomically disadvantaged region (Abstract 1030). The overall incidence of virologic failure, defined as an HIV RNA level above 1000 copies/mL after 6 months of a second antiretroviral regimen, was 21.5 per 100 person-years, with a cumulative incidence of 45% by 5 years. A modified medication possession ratio during initial and second regimens, calculated as the number of months for which a refill was submitted as a fraction of the number of months in the treatment period, was statistically significantly inversely correlated with virologic failure. The concern that nonadherence to initial and second regimens predicts virologic failure during second regimens is particularly important in low- and middle-income countries in which there is a move to increase access to initial antiretroviral regimens but in which options for third regimens are limited.

The Impact of Changing CD4+ Cell Count Thresholds. Numerous changes over the past decade in WHO and other guidelines regarding CD4+ cell count eligibility for antiretroviral therapy culminated in the most recent 2015 WHO guidelines recommending universal HIV testing and antiretroviral therapy for all individuals living with HIV infection.[13] Several groups of investigators explored the impact of changing CD4+ cell count thresholds on programs in low- and middle-income countries.

Bor and colleagues used data from the Hlabisa HIV Treatment and Care Programme in South Africa and a regression discontinuity design to estimate the impact of immediate versus delayed antiretroviral therapy for failure to meet the CD4+ cell count eligibility set point of less than 350/μL in a real-world setting (Abstract 1011). The investigators found that retention in care at 12 months was statistically more likely in those with an eligible CD4+ cell count of just below 350/μL (50%) than in those with a CD4+ cell count slightly above the threshold (32%; $P = .001$). Those with an eligible CD4+ cell count were also statistically significantly more likely to initiate antiretroviral therapy within 6 months (43% vs 18%, respectively) and to be retained in care at 18 months to 24 months. The regression discontinuity design implies that the groups are otherwise demographically and biologically similar, and these data suggest that initiation of antiretroviral therapy has a large impact on long-term retention in HIV care.

Bor and colleagues also used data from the Hlabisa cohort to inform a mathematical model to determine the number of new initiations of antiretroviral therapy South Africa could expect if a universal test-and-treat strategy were adopted nationally and CD4+ cell count thresholds for eligibility were eliminated (Abstract 1048). An estimated 40% of participants presented for treatment with CD4+ cell counts higher than 500/μL. Although 8% of those with CD4+ cell counts at or above 350/μL initiated therapy despite being over the current eligibility threshold, only 40% of those with CD4+ cell counts at or below 500/μL initiated therapy. Based on these data, the investigators estimated that 72.8% of individuals will not initiate treatment despite being eligible for it and that elimination of treatment eligibility thresholds based on CD4+ cell count will not help achieve 90-90-90 targets.

Kluberg and colleagues examined the impact of the 2011 South African Ministry of Health policy change that extended eligibility for antiretroviral therapy from those with CD4+ cell counts below 200/μL to those with counts below 350/μL on treatment delays among those with counts below 200/μL, to determine whether eligibility expansions adversely impact more immunocompromised patients (Abstract 1012). Across 17 rural clinics in KwaZulu-Natal, the rate of initiation of antiretroviral therapy increased among the newly eligible

(60% in those with CD4+ cell counts of 200/μL-349/μL; 95% CI, 20%-220%) and the previously eligible (20% in those with CD4+ cell counts < 200/μL; 95% CI, 0%-40%) in the 12 months after eligibility expansion. The investigators noted no short-term negative impact of eligibility expansion, based on the stable or increasing rates of initiation of antiretroviral therapy among those with low CD4+ cell counts.

Models for Improved Care Delivery in Low- and Middle-Income Countries

Expediting Initiation of Antiretroviral Therapy. Several well-designed randomized controlled trials and programmatic studies examined different strategies for expediting initiation of antiretroviral therapy, which should increase retention in care and decrease mortality. Rosen and colleagues presented data from the RapIT (Rapid Initiation of Antiretroviral Therapy to Promote Early HIV/AIDS Treatment in South Africa) trial, a randomized control trial of same-day treatment initiation in Johannesburg, South Africa (Abstract 28). Participants were randomly assigned to same-day treatment initiation (at time of HIV diagnosis or at first presentation to clinic if previously diagnosed) or standard of care. The trial took place in an outpatient primary care clinic and a hospital-based HIV treatment center and utilized point-of-care laboratory testing to allow immediate treatment eligibility assessment for the arm that received rapid treatment. Initiation of antiretroviral therapy within 90 days of randomization, the primary outcome measure, occurred in 72% of those in the arm that received the standard of care (n = 229) and 97% of those in the arm that received rapid treatment (n = 234; RR, 1.36; 95% CI, 1.24, 1.49). Those in the arm that received rapid treatment were also more likely to be virologically suppressed (HIV RNA level ≤400 copies/mL) at 10 months after randomization than those that received the standard of care (RR, 1.26; 95% CI, 1.05, 1.50). Other notable aspects of rapid initiation were that 4 of the 5 participants who were lost to follow-up in that arm were lost during workup for tuberculosis and that 75% initiated antiretroviral therapy on the same day, with a median time in clinic of 2.4 hours. The investigators concluded that it is possible to start nearly all patients on antiretroviral therapy within 1 month of HIV diagnosis or presentation to care, but it should be noted that the staff assigned to the arm that received rapid initiation of treatment were all research staff. Further data on how this intervention would be received in a nonresearch setting would be useful.

Investigators examined different strategies for expediting initiation of antiretroviral therapy, which should increase retention in care and decrease mortality.

Amanyire and colleagues shared findings from a stepped wedge cluster randomized trial of an intervention to reduce delays in initiation of antiretroviral therapy across 20 clinics in Uganda (Abstract 112). The intervention was implemented in randomly selected groups of 5 clinics every 6 months and included opinion leader–led teaching and coaching for practitioners, a revised counseling protocol to link adherence counseling to initiation of treatment, real-time CD4+ cell counts to assess eligibility for treatment, and regular feedback to clinics regarding delays in initiation of treatment. Overall, 12,024 individuals met eligibility criteria, although the threshold for initiation of treatment changed from a CD4+ cell count of 350/μL or below to a count of 500/μL or below during the course of study. The investigators found that in intervention clinics, 79.6% of individuals initiated treatment within 14 days of eligibility, whereas only 37.7% did so in the control group (RR, 2.11, 2.03, 2.20; $P < .0001$). There was also a statistically significant difference in the number of individuals who had initiated treatment at 90 days after eligibility (89.7% in the intervention group and 70.4% in the control group; RR, 1.27; 95% CI, 1.20, 1.30; $P < .0001$). The percentage of individuals who achieved an HIV plasma RNA level of less than 200 copies/mL 1 year after eligibility was assessed in a random sample of 437 individuals; there was no statistically significant difference between the 2 groups, unless those individuals who were missing HIV RNA measurements were excluded. There was also no statistically significant difference in adherence to medical visits between the 2 groups. The strength of this study is that the intervention is embedded within existing HIV treatment programs, which increases external generalizability, and it is hoped that further analyses of these data will show the long-term benefits of early initiation of antiretroviral treatment, which were not seen in the analysis thus far.

Hoffmann and colleagues conducted a 4-arm randomized controlled trial in Johannesburg, South Africa, of accelerated entry into HIV care after diagnosis via mobile HIV counseling and testing units, from which entry into care is often lower than when testing occurs in clinical settings (Abstract 113LB). After receiving a diagnosis of HIV infection, 2558 individuals were randomly assigned to 1 of 4 arms: 1) standard of care; 2) point-of-care CD4+ cell count testing to assess eligibility for antiretroviral therapy; 3) point-of-care CD4+ cell count testing plus care facilitation using the CDC Antiretroviral Treatment and Access to Services (ARTAS) approach; and 4) point-of-care CD4+ cell count testing plus reimbursement for transportation. The investigators did not find any statistically significant difference in the primary outcome of self-reported entry into care at 90 days between the arms, but the secondary outcome of documented entry into care at 90 days was statistically significantly more likely in the arm that included care facilitation: 38% in the arm that received care facilitation compared with 29% in the arm that received the standard of care (HR, 1.4; 95% CI, 1.1, 1.7). The same association was seen for the secondary outcome of initiation of treatment by 180 days. Only 30% of participants were eligible for treatment, but 18% of those in the arm that received care facilitation initiated treatment compared with 13% in those who received the standard of care, or more than half of those who were eligible (HR, 1.4; 95% CI, 1.1, 1.9). No statistically significant differences were seen between standard of care and the 2 other

intervention arms (point-of-care CD4+ cell count testing and point-of-care CD4+ cell count testing plus reimbursement for transportation) for any of the secondary outcomes. Hoffman noted that other studies have also shown overreporting of engagement in care, when documentation is not required, and that outcome ascertainment for engagement in care may be essential to measuring the true impact of a program. The increased contact with patients at 30 days and 60 days after testing, included even in the arm that received the standard of care, may have led to higher rates of engagement in care than those seen in traditional mobile testing programs, but many challenges remain in achieving the 90-90-90 targets for those diagnosed with HIV infection in non–clinic-based settings.

Kwarisiima and colleagues presented virologic outcomes of a streamlined care model for people living with HIV infection with CD4+ cell counts above 350/μL in rural Kenya and Uganda (Abstract 116). The streamlined model involved a patient-centered approach to care, including empathetic handling of adherence and retention issues; efficient visits with rapid 1-day to 3-day waits for initiation of antiretroviral treatment, minimal wait times, 3-month medication supplies, and nurse triage for follow-up visits; viral load counseling structured by treatment status; access to clinicians via telephone; and appointment reminders by phone or text messaging. Of 972 enrolled participants, 86% were retained in care and had an HIV RNA measurement at 48 weeks after initiation of treatment. Retention in care at 48 weeks was 90%, with no difference by CD4+ cell count strata (350/μL-500/μL compared with >500/μL). Similarly, 93% of participants had an HIV RNA level of less than 500 copies/mL, with no difference by CD4+ cell count strata. If individuals without a 48-week HIV RNA measurement, for any reason (eg, loss to follow-up, death, or study withdrawal), were classified as virologic failures, 87% of participants had an HIV RNA level below 500 copies/mL. Adverse events and regimen switches were also minimal. The investigators recommended this type of streamlined care for delivery of antiretroviral therapy to asymptomatic patients with high CD4+ cell counts.

Differentiated Models of HIV Care Delivery. Differentiated HIV care, as defined by Grimsrud (Abstract 122), is "the continuum of adaptations that can be made to HIV services with the intention of streamlined care, including [antiretroviral therapy] delivery. The objective is to provide quality, patient-centered care reflecting the preferences and expectations of [persons living with HIV infection], while reducing unnecessary burdens on the health system." Grimsrud provided an overview of various differentiated care models, and the following abstracts presented new data on potential ways to differentiate HIV care to improve outcomes and efficiency.

Roy and colleagues evaluated opportunities for streamlining care by examining visit burden and appointment patterns for stable patients taking antiretroviral therapy with CD4+ cell counts above 500/μL or above 350/μL (Abstract 1018). The investigators found that 58% of all visits were for pharmacy refills or adherence counseling without a clinical assessment and that median appointment intervals remained stable at 60 days, regardless of years taking antiretroviral therapy or CD4+ cell count. The investigators proposed that, given the growing proportion of visits (23% currently and rising over time) made by those taking antiretroviral therapy for more than 6 months with a CD4+ cell count above 500/μL, who might be considered clinically stable, increased visit spacing for these individuals or differentiated care in the form of community adherence clubs or other community-based treatment models would be feasible.

Sharp and colleagues explored the feasibility of expanding differentiated care to clinically unstable individuals who recently achieved virologic suppression after a documented treatment failure (Abstract 1031), a departure from traditional models in which only highly clinically stable individuals are considered for differentiated or de-escalated care. In Khayelitsha, South Africa, individuals with 2 consecutive HIV RNA measurements above 200 copies/mL were referred to a lay health care worker–led group support session and adherence consultation with a nurse. Participants who achieved an HIV RNA level of less than 400 copies/mL after this intervention were then referred to adherence clubs for care. In this context, adherence clubs were groups of approximately 30 individuals led by lay health care workers meeting 5 times per year for peer support, brief symptoms screening, and supply of antiretroviral medications, supplemented by an annual visit with a clinician and HIV RNA measurement. After 18 months of follow-up, 89% of 165 participants were still retained in care, and 81% remained in the adherence club model. Of note, 78% still had viral suppression (HIV RNA level <400 copies/mL) at 18 months, arguing that even those who had recently met criteria for virologic failure could achieve positive long-term outcomes through differentiated care in adherence clubs. This strategy is particularly encouraging, as it could increase capacity for antiretroviral therapy in low- and middle-income countries.

Geng provided a summary of how to incorporate various potential interventions to improve retention in care programs in low- and middle-income countries (Abstract 121). Geng emphasized that retention in care is the driver of efficiency and effectiveness in the global antiretroviral therapy response but that barriers to retention in care often vary in intensity, nature, and effects. In a meta-analysis of individual-level data on 12,765 individuals in 26 tracing studies, 3 main categories of barriers were identified: structural, such as transportation or financial; psychosocial, such as stigma and family support; and clinic based. Comparison of those who discontinued care with those who transferred to other clinics without informing their practitioner within the IeDEA cohort found that psychosocial barriers were more commonly reported in those disengaged from care (76% vs 27%) but that clinic-based barriers

Retention in HIV care is the driver of efficiency and effectiveness in the global antiretroviral therapy response, but barriers to retention in care often vary in intensity, nature, and effects.

were more commonly reported by those who transferred clinics without informing their practitioner (33% vs 15%), with both of these differences being statistically significant. These data suggest that not all loss to follow-up is similar, and certain barriers predict lasting discontinuation more successfully. Geng argued for a personalized public health approach in which individual responses, such as those elucidated in a simple survey but that were predictive of mortality (Bassett and colleagues, Abstract 1016, described above), could dictate specific interventions if measured appropriately. Combinations of strategies could comprise a prevent-and-treat retention strategy in which intensive interventions, such as patient navigation, microclinics, or patient-empowerment initiatives, would be initially implemented and then followed by de-escalation to less intensive interventions, such as 2-way text messaging reminders. Alternatively, an induction-maintenance strategy could be employed in which low-intensity interventions are initially offered and higher-intensity interventions are applied only to those who do not remain engaged in care. Geng proposed that implementation science techniques be used to systematically collect sufficient programmatic data to measure the efficacy of various stacked interventions, rather than relying on more expensive and often impractical, sequential multiple assignment randomized controlled trials or other more traditional research techniques.

Antiretroviral Treatment in the United States: Life Expectancy, Care Cascade Outcomes, and Strategies to Improve the HIV Care Continuum

Bradley and colleagues used data from 2009 to 2013 collected by the US Medical Monitoring Project (MMP), a national surveillance system sampling data from 23,125 participants across 23 jurisdictions, to estimate the proportion of individuals in care who achieved virologic suppression, defined as having an HIV RNA level below 200 copies/mL at the last test and at all tests over the prior 12 months (Abstract 53). Increases in virologic suppression were observed over time, from 72% in 2009 to 80% in 2013 ($P < .01$, for trend); however, these gains were less pronounced among women, those aged 18 years to 29 years or 30 years to 39 years, and those of black race. The largest increase in sustained virologic suppression over the past 12 months occurred among those aged 18 years to 29 years (32% in 2009 to 51% in 2013; ($P < .01$, for trend). MSM also consistently had higher than average rates of virologic suppression at last test and sustained virologic suppression. Although there were increases in the number of individuals taking antiretroviral therapy (89% in 2009 to 94% in 2013), the rate of change in sustained virologic suppression was higher over the same time period (58% to 68%), implying that although increases in the number of people taking antiretroviral therapy contribute to this improvement, advances in antiretroviral therapy that make it more tolerable and easier to take also impact virologic suppression. The investigators cited substantial changes in national policy, such as the recommendation for universal antiretroviral treatment for everyone living with HIV infection and the elimination of wait lists in the AIDS Drug Assistance Program, as important contributing factors to improvements in virologic suppression.

Encouraging data regarding continued improvements in life expectancy for people living with HIV infection were presented by Marcus and colleagues (Abstract 54). HIV-infected individuals receiving care within the Kaiser Permanente California system from 1996 to 2011 were matched 10:1 with uninfected individuals within the same system by age, sex, medical center, and year. Mortality rates for people living with HIV infection over this time period decreased from 7077 per 100,000 person-years in 1996 to 1054 per 100,000 person-years in 2011, whereas mortality for uninfected individuals remained stable (439/100,000 person-years in 1996 to 381/100,000 person-years in 2011). Similarly, life expectancy at age 20 years for uninfected individuals remained stable (63 years in 1996 to 65 years in 2011) but increased dramatically (19 years in 1996 to 53 years in 2011) for people living with HIV infection. Many of the disparities seen in national data for people living with HIV infection were evident in this insured cohort with access to care.[14] Life expectancy was lowest among those of black race ($P = .007$, compared with those of white race) and those reporting the HIV transmission risk behavior of injection drug use ($P = .011$, compared with those in whom heterosexual sex was their risk behavior). Despite dramatic improvements in life expectancy for people living with HIV infection, it is concerning that in this cohort of insured individuals who are engaged in medical care, a 13.1-year gap (95% CI, 11.5, 14.6) remains between the HIV-infected and uninfected populations. This gap narrowed to 7.9 years for those living with HIV infection who initiated antiretroviral therapy at CD4+ cell counts at or above 500/μL. These data imply that more work is needed to ensure normal life spans for persons living with HIV infection and that early initiation of antiretroviral therapy and addressing health disparities may play a substantial role in achieving this goal.

Despite dramatic improvements in life expectancy for people living with HIV infection, a 13.1-year life expectancy gap remained between persons living with HIV infection and the uninfected population.

Yaylali and colleagues described the impact of improving HIV care and treatment and initiating PrEP in the United States. A compartmental model was used to assess the marginal benefit of adding PrEP to improvements in the existing care cascade (Abstract 1051). These data are reviewed in more detail by Buchbinder and Liu.[15]

Metsch and colleagues presented the results of a study seeking to improve engagement in care for a key population: persons who use alcohol excessively or who use recreational drugs. The HOPE (Hospital Visit as an Opportunity for Prevention and Engagement) study targeted individuals hospitalized for substance use with HIV RNA levels above 200 copies/mL and CD4+ cell counts at or below 500/μL (Abstract 27). The investigators randomly assigned 801 such individuals across

11 US hospitals to 1 of 3 arms: treatment as usual; 6 months of patient navigation; or patient navigation with contingency management, a series of conditional financial incentives providing up to $1160 for HIV care or substance use treatment visits, medication pickups, achievement of virologic suppression (HIV RNA ≤200 copies/mL), and drug-free toxicology screening results. The primary outcome was achievement of virologic suppression at 12 months, and there was no statistically significant difference in this outcome by treatment arm at 12 months: 34.1% with treatment as usual, 34.1% with 6 months of patient navigation, and 38.6% with patient navigation plus contingency management ($P = .68$). However, there was a statistically significant difference in virologic suppression at 6 months between the arm that received treatment as usual (33.6%) and the arm that received patient navigation plus contingency management (46.2%; $P = .04$). There were also statistically significant differences at 6 months, although not at 12 months, in the secondary outcome of visit to an HIV practitioner, with a benefit observed in the intervention arms; no differences were observed in rehospitalizations, death, or engagement in substance use treatment. Also of interest, the 4 sites in the Southern United States had significantly lower rates of virologic suppression at 12 months (24.1%-26.0%) than all other sites (36.4%-60.9%; $P < .0001$). Individuals of black race and those who used stimulants were also statistically significantly less likely to achieve virologic suppression than those of white race and those who used opioids, respectively. The investigators concluded that these populations are difficult to reach and that, considering the important findings after 6 months of patient navigation, a sustained intervention may be necessary for continued benefit.

Prevention and Treatment of Pediatric HIV Infections

Option B+ was a program implemented in Malawi in 2011 to offer lifelong antiretroviral therapy to all HIV-infected pregnant and breastfeeding women irrespective of their clinical or immunologic status. Begun in 2014, the NEMAPP (National Evaluation of Malawi's PMTCT Program) study applied a stratified, multistage cluster sampling schema to identify a nationally representative cohort of HIV-exposed infants aged 4 weeks to 26 weeks and their mothers to measure the effects of Option B+ on MTCT rates. Gupta (Abstract 35LB) presented data focused on the cohort of infants aged 4 weeks to 12 weeks in the NEMAPP study. Mothers were screened for HIV infection, and HIV-exposed infants were tested for HIV infection using HIV DNA PCR assays. Among 1851 HIV-infected mothers of infants aged 4 weeks to 12 weeks, antiretroviral therapy coverage was very high at 93.5% and overall MTCT was very low at 4.1%. For those who took antiretroviral therapy during pregnancy, MTCT was even lower at 2.9%. For the 6.5% of women who never started treatment at any time during pregnancy or immediately postpartum, MTCT was higher at 20.3%. The MTCT rate differed by timing of treatment initiation, ranging from 1.4% in women who initiated treatment prior to pregnancy to approximately 4% in women who initiated treatment during pregnancy.

Current US and WHO guidelines recommend TDF as a preferred antiretroviral drug for HIV-infected pregnant women.[13,16] However, there are concerns about the adverse effects of TDF on bone development, including effects on fetal bone mineral content (BMC) as a result of exposure to maternal TDF. Siberry and colleagues (Abstract 36) presented data from the P1084s substudy of the International Maternal, Pediatric, Adolescent AIDS Clinical Trials (IMPAACT) Network PROMISE (Promoting Maternal-Infant Survival Everywhere) trial. The substudy compared newborn bone mineral content, measured using dual-energy X-ray absorptiometry (DXA) scans of whole body and lumbar spine obtained within 28 days of birth, in 359 infants by exposure to maternal antiretroviral regimens at gestational ages older than 14 weeks at 8 sites in 4 African countries. The women were randomly assigned to initiate 1 of 3 antiretroviral regimens during pregnancy: arm 1) zidovudine with single-dose nevirapine plus TDF and emtricitabine; arm 2) zidovudine, lamivudine, and ritonavir-boosted lopinavir; or arm 3) TDF, emtricitabine, and ritonavir-boosted lopinavir. No adverse associations were found between infant whole-body and lumbar spine BMC measurements and maternal TDF use when arms 2 and 3 were directly compared. This is in contrast to a finding from a published US cohort study that reported a 12% lower mean newborn BMC level following maternal TDF use.[17] The PROMISE substudy also found that initiation of a triple-drug antiretroviral regimen that included boosted lopinavir during pregnancy (arms 2 and 3 compared with arm 1) was associated with lower mean whole-body BMC levels, even after adjustment for maternal and infant factors. An analysis exploring mediating factors for this finding is underway.

HIV infection has negative effects on bone accrual in children, with decreased peak bone mass and increased risk of osteoporosis and fracture later in life. Data are lacking, however, on ways to optimize antiretroviral therapy to improve bone health in children. In Abstract 40, Arpadi and colleagues examined whether preemptive switching of an initial ritonavir-boosted lopinavir–based ART to efavirenz, compared with continuing on the regimen, was associated with improved bone outcomes in HIV-infected children aged 5 years to 10 years in South Africa. The clinical trial randomly assigned 113 HIV-infected children to the efavirenz-containing arm and 106 children to the boosted lopinavir–containing arm; the children also received 2 nRTIs, excluding TDF. In addition, the study enrolled 180 HIV-uninfected children as controls. The BMC *z* score was lower in HIV-infected children than in uninfected children, even after adjustment for dietary calcium and vitamin D intake and activity level. The BMC *z* score was lower among those taking ritonavir-boosted lopinavir than those taking efavirenz (-1.07 vs -0.49; $P < .001$), and this difference persisted even after adjustment for various factors, including physical activity, dietary calcium and vitamin D intake, viral load, and CD4+ cell count. BMC *z* score was higher with increased duration on efavirenz since switch from boosted lopinavir. The investigators concluded that in addition to avoiding the adverse effects associated with boosted

lopinavir, such as dyslipidemia and lipodystrophy, switching to efavirenz offers a benefit of improved bone mass accrual in HIV-infected children.

Several studies in developing countries have shown that HIV-exposed uninfected children have higher rates of mortality compared with HIV-unexposed children, for reasons yet to be elucidated. Treatment with cotrimoxazole has been demonstrated to decrease mortality in HIV-infected children, although data from randomized clinical trials on its efficacy among HIV-exposed uninfected children are lacking. Shapiro and colleagues conducted a randomized, double-blinded study of 2848 HIV-exposed uninfected children in a nonmalarial region of Botswana who received cotrimoxazole (n = 1423) or placebo (n = 1425) from 14 days to 34 days of life through 15 months of life (Abstract 37). Follow-up visits occurred every 1 month to 3 months through 18 months, with those infants diagnosed with HIV infection after random referral to open-label cotrimoxazole. Infants were fed by formula or breastfeeding based on the preference of the mother. The data and safety monitoring board stopped the study early due to futility in showing a benefit of cotrimoxazole. Mortality rates at 18 months did not differ between the arm that received cotrimoxazole and the arm that received placebo (2.4% vs 2.64%; $P = .7$). No differences were detected between the arms for the secondary outcomes, including hospitalization, grade 3 or 4 HIV diagnosis, or grade 3 or 4 anemia, whereas grade 3 or 4 neutropenia was more common in the group that received cotrimoxazole.

A randomized trial comparing urgent with poststabilization antiretroviral therapy was conducted among 183 hospitalized antiretroviral therapy–naive, HIV-infected children aged 0 years to 12 years in 4 Kenyan hospitals (Abstract 38), to evaluate whether urgent antiretroviral therapy with immune reconstitution leads to improved or worse outcomes. In the study, which was stopped early during interim analysis by the data safety and monitoring board due to futility, the children in the urgent-treatment arm (n = 90) received antiretroviral treatment within 48 hours (median, 1 day), and those in the poststabilization-treatment arm (n = 93) received antiretroviral treatment within 7 days to 14 days (median, 8 days). A notable baseline difference included lower CD4+ cell counts in the urgent-treatment arm compared with the poststabilization-treatment arm (12.5% vs 17%; $P = .02$). Overall mortality was 61 per 100 person-years, Mortality was 69 per 100 person-years in the urgent-treatment arm compared with 53 per 100 person-years in the poststabilization-treatment arm (HR, 1.46; 95% CI, 0.67, 2.37; $P = .47$), and no difference in mortality was noted after adjustment for baseline CD4+ cell count. Additionally, no statistically significant differences in rates of IRIS and serious adverse events were detected between the 2 arms.

In hospitalized HIV-infected Kenyan children, no difference in rates of mortality, IRIS, or serious adverse events was noted between urgent initiation of antiretroviral therapy (within 48 hours) and poststabilization antiretroviral therapy (within 7-14 days).

Clinical Pharmacology and Modeling Predictions of Antiretroviral Drugs in Pregnancy and Pediatric Populations

A Themed Discussion session (Session TD-1) focused on clinical pharmacology and modeling predictions of antiretroviral drugs in pregnancy and pediatric populations. Decreased exposure to many antiretroviral drugs occurs during pregnancy as a result of physiologic variations and altered pharmacokinetics. IMPAACT P1026s is an ongoing open-label, phase IV prospective study of the pharmacokinetics and safety of antiretroviral drugs, including dolutegravir and rilpivirine, in HIV-infected pregnant women. Data on the pharmacokinetics of dolutegravir during pregnancy and postpartum as well as infant washout pharmacokinetics and safety are lacking. Mulligan and colleagues analyzed the 24-hour pharmacokinetics of dolutegravir 50 mg administered once daily to 15 HIV-infected pregnant and postpartum women in the United States as part of the IMPAACT P1026s study and examined safety and infant washout pharmacokinetics (Abstract 438). The analysis encompassed data from 9 women in their second trimester, 15 in their third trimester, and 9 at 6 weeks to 12 weeks postpartum; 10 infants were included in the infant washout analysis. The dolutegravir area under the curve (AUC) was 25% to 30% lower in the second and third trimesters of pregnancy compared with paired postpartum levels, and dolutegravir plasma concentration at 24 hours after administration was 41% lower during pregnancy than in paired postpartum values. These differences, however, were not statistically significant, and all 15 women were noted to have an undetectable viral load at the time of delivery. Moderate elevation of alanine aminotransferase levels, possibly related to dolutegravir, was detected in 1 pregnant woman, and 2 women experienced preeclampsia. All 9 infants who were tested were found to be HIV-uninfected. The elimination half-life of dolutegravir in infants was 34.5 hours, more than twice that observed in nonpregnant adults as well as in pregnant and postpartum women in this study. Congenital abnormalities were reported in 4 infants, and 4 experienced hypoglycemia. The investigators concluded that more data on dolutegravir pharmacokinetics, safety, and outcomes in pregnant women and infants are warranted before dolutegravir can be recommended for treatment during pregnancy.

Rilpivirine is a second-generation NNRTI being investigated as a potential drug for PrEP in HIV-uninfected women and for prevention of intrapartum MTCT in HIV-infected women. Using data from the IMPAACT P1026s cohort, Best (Abstract 439) presented results of a study examining concentrations of rilpivirine in the female genital tract in 24 HIV-infected pregnant and postpartum women who received rilpivirine 25 mg once daily orally as part of their care. Plasma and cervicovaginal fluid specimens were collected and measured

for rilpivirine concentrations at the following intervals during the second and third trimesters of pregnancy and at 6 weeks to 12 weeks postpartum: before the first dose and then 1 hour, 2 hours, and 4 hours after the first dose. For all samples combined, median rilpivirine concentration was 92 ng/mL (IQR, 49-147) in plasma and 70 ng/mL (IQR, 23-121) in cervicovaginal fluid, which is at least 100 times more than the protein-free 90% effective concentration for rilpivirine of 0.66 ng/mL, considered the inhibitory concentration. The investigators found that the AUC of rilpivirine in cervicovaginal fluid was similar to that in plasma over the first 4 hours of dosing during the second and third trimesters of pregnancy; however, the AUC of rilpivirine in cervicovaginal fluid was lower postpartum than during pregnancy. The investigators suggested that rilpivirine concentrations are likely to reach inhibitory concentrations in the female genital tract when the drug is administered orally and that concentrations of drug in cervicovaginal fluid may be higher during pregnancy than postpartum.

The final 2 abstracts presented in Session TD-1 pertained to pharmacokinetic modeling of antiretroviral drug clearance in pregnant women and infants. Clearance of nevirapine occurs primarily via hepatic metabolism by cytochrome P450 (CYP450) enzymes 2B6 and 3A4 (CYP2B6 and CYP3A4) and secondarily via renal excretion. Nevirapine clearance is reduced in term and premature infants because of immature CYP450 enzyme activity. Autoinduction of nevirapine clearance occurs in adults and older children, but its extent in infants is unclear. Data on the effects of immaturity of CYP450 enzyme activity and autoinduction on nevirapine clearance are crucial for selecting an optimal nevirapine dosing regimen in infants.

In Abstract 440, Mirochnick and colleagues developed a population model of existing nevirapine pharmacokinetic data and simulations to examine dosing regimens of nevirapine 6 mg/kg twice daily for term infants and nevirapine 4 mg/kg twice daily for 1 week followed by 6 mg/kg twice daily for late-term infants of 34 weeks to 37 weeks gestation to achieve a treatment target trough concentration of at least 3 µg/mL. Data were collected from 192 infants younger than 1 year who were enrolled in 1 of 5 Pediatric ACTG and HIV Prevention Trials Network protocols in the United States, Brazil, and Africa. The investigators employed software to model changes in nevirapine pharmacokinetics over time, with imputation of the effects of immaturity of CYP2B6 and CYP3A4 enzymes and rate of autoinduction on nevirapine clearance from published data. The model found that nevirapine clearance was low immediately following birth and increased substantially during the first 2 months of life. In addition, autoinduction of nevirapine clearance was proportional to the nevirapine dose size during the first 12 months of life. Nevirapine clearance was also influenced by CYP3A4 metabolism and prematurity status. The simulations confirmed that the 2 nevirapine dosing regimens achieved the treatment target trough level of at least 3 µg/mL, and these 2 regimens are currently being evaluated in the IMPAACT 1115 and 1006 studies.

In Abstract 441, Olagunju and colleagues presented results of physiologically based pharmacokinetic modeling to simulate and predict infant exposure to maternal therapeutic efavirenz from breast milk during lactation. The investigators developed a model combining whole-body physiologically based pharmacokinetic maternal and infant models and incorporated system- and drug-specific parameters for absorption, distribution, metabolism, excretion, and breastfeeding. The model involved virtual populations of breastfeeding mother-infant pairs (n = 100 per infant age group) and assumed that the mothers were receiving efavirenz 600 mg orally daily and that infants were breastfed exclusively for the first 6 months of life. The model was found to sufficiently describe efavirenz pharmacokinetics in plasma and breast milk, with prediction of breast milk, plasma pharmacokinetic parameters, and infant exposure being within 50% difference compared with clinical data. The investigators concluded that this modeling approach sufficiently predicted infant exposure to maternal therapeutic efavirenz from breast milk and emphasized that physiologically based pharmacokinetic modeling can be applied and expanded to studies of exposure to other maternal drugs during lactation.

The use of long-acting antiretroviral drugs for HIV treatment is a promising strategy among children and adolescents, offering the potential benefits of regimen simplification, cost savings, and improvement in adherence. Rajoli and colleagues applied physiologically based pharmacokinetic modeling in their study to assess the in vivo pharmacokinetics of a long-acting injectable formulation of cabotegravir and rilpivirine in children and adolescents and to ascertain appropriate dosing regimens (Abstract 442). The models incorporated in vitro pharmacokinetic data for cabotegravir and rilpivirine and were validated against published clinical data on long-acting formulations of the 2 drugs in adults. Simulations were performed for 200 virtual pediatric patients for each weight band between age 3 years and 20 years following intramuscular injections of cabotegravir and rilpivirine. The models factored in demographics, tissue size, and drug-specific parameters, including metabolism and distribution. The simulated pharmacokinetics for long-acting cabotegravir and rilpivirine in adults in the models were consistent with available clinical data. More specifically, for cabotegravir 800 mg administered intramuscularly on a quarterly basis, mean AUC was 4467 µg.h/mL and 5257 µg.h/mL, maximum concentration (C_{max}) was 3.3 µg/mL and 3.54 µg/mL, and trough concentration (C_{trough}) was 1.1 µg/mL and 1.2 µg/mL for children and adolescents and for adults, respectively. For rilpivirine 900 mg administered intramuscularly on a monthly

A long-acting injectable formulation of cabotegravir and rilpivirine adjusted for weight may be a potentially valuable dosing strategy in children and adolescents, offering benefits of regimen simplification, cost savings, and improvement in adherence.

basis, mean AUC was 74,420 ng.h/mL and 91,087 ng.h/mL, C_{max} was 168 ng/mL and 168.7 ng/mL, and C_{trough} was 79.1 ng/mL and 78.3 ng/mL for children and adolescents and for adults, respectively. The models predicted optimized doses of cabotegravir and rilpivirine administered quarterly and monthly, respectively, for all weight bands, with at least 95% of individuals achieving C_{trough} levels over the cutoff limits. The investigators concluded that long-acting injectable cabotegravir and rilpivirine adjusted for weight could be a potentially valuable dosing strategy for children and adolescents.

New Antiretroviral Drugs and Approaches for Treatment of Pediatric HIV Infection

Data on new antiretroviral drugs for the treatment of HIV-infected pediatric populations were presented in a themed discussion session (Session TD-10). Nevirapine dosing in infants for PMTCT has been well studied; however, data are lacking on optimal nevirapine dosing for treatment of HIV infection in newborn infants. Capparelli and colleagues presented results of a study that assessed safety and drug concentrations of nevirapine 6 mg/kg twice daily for the treatment of HIV-infected newborn infants (Abstract 815). Six infants born at or later than 35 weeks gestation who weighed 2 kg or more were treated with nevirapine 6 mg/kg twice daily along with lamivudine 2 mg/kg twice daily and zidovudine 4 mg/kg twice daily until 2 weeks of age or for 40 weeks gestational age, whichever occurred later. A decrease in HIV RNA was detected in all infants. No antiretroviral therapy–related adverse events were reported during the first month. At week 2, nevirapine drug concentrations ranged from 3 mcg/mL to 11 mcg/mL and were considered therapeutic levels. Based on these findings, the investigators recommended nevirapine 6 mg/kg twice daily for HIV treatment in term and near-term infants.

Data from the IMPAACT P1093 study of dolutegravir pharmacokinetics, safety, and dosing in children and adolescents were presented by Wiznia and colleagues (Abstract 816). Twenty-three treatment-experienced, InSTI-naive children between age 6 years and 12 years with an HIV RNA level at or above 1000 copies/mL were enrolled in this ongoing phase I/II open-label study. Eleven children were involved in stage 1 with intensive pharmacokinetics, and 12 children were involved in stage 2 with no pharmacokinetics, safety, and efficacy. In stage 1, dolutegravir was added to a failing antiretroviral regimen (monotherapy phase) with an optimized background regimen after intensive pharmacokinetics at day 5 to day 10 (continuation phase). In stage 2, dolutegravir plus an optimized background regimen was initiated at study entry. The primary outcome of virologic success was defined as an HIV RNA below 400 copies/mL by week 48; the secondary outcome was an HIV RNA below 50 copies/mL by week 48. The median age of the children was 10 years (range, 6-11 years). The baseline median CD4+ cell count was 645/µL (range, 466/µL-732/µL), and the baseline CD4+ cell percentage was 24% (14%-29%). Virologic success, defined as achieving an HIV RNA level below 400 copies/mL was observed in 78.3% (95% CI, 56.3%-92.5%) of the children. Increases in CD4+ cell count of 387/µL (range, 49/µL-575/µL) and in CD4+ cell percentage of 9% (7%-14%) were seen. Dolutegravir was well tolerated by the children, with no drug discontinuations because of adverse events and no severe dolutegravir-related clinical or laboratory adverse events. The investigators concluded that treatment with dolutegravir in addition to an optimized background regimen in children aged 6 years to 12 years was well tolerated and led to high rates of virologic suppression and immunologic recovery at 48 weeks.

TDF has been associated with bone and renal toxic effects. TAF is a prodrug of tenofovir that is metabolized intracellularly to tenofovir and phosphorylated to active TDF and, therefore, leads to 91% lower exposure to tenofovir in plasma than TDF. Hence, TAF 10 mg coformulated in a once-daily single-tablet regimen with elvitegravir 150 mg, cobicistat 150 mg, and emtricitabine 200 mg has the potential for better bone and renal safety and for increased antiretroviral adherence in adolescents.

Gaur and colleagues presented results from a phase II/III single-arm, open-label study on the safety, efficacy, and steady-state pharmacokinetics of TAF, elvitegravir, cobicistat, and emtricitabine in HIV-infected adolescents through 48 weeks (Abstract 817); pharmacokinetic data through 24 weeks were previously reported.[18] Fifty HIV-infected, treatment-naive adolescents between age 12 years and 18 years with an HIV RNA level at or above 1000 copies/mL, a CD4+ cell count above 100/µL, and weight at or above 35 kg were enrolled in the study. Two adolescents discontinued the study before week 48. The primary end point for this analysis was virologic success, defined as an HIV RNA level of less than 50 copies/mL at week 48. Forty-six participants (92%) achieved virologic success; the mean increase in CD4+ cell count was 224/µL. No antiretroviral drug resistance mutations were detected. Six participants (12%) experienced serious adverse events, one of which was study drug–related and resolved without drug discontinuation. No cases of proximal renal tubulopathy were reported. The most common drug-related adverse events included nausea, abdominal pain, and vomiting. The median (IQR) change in serum creatinine level at week 48 compared with baseline was an increase of 0.07 mg/dL (0.02, 0.15), which was attributed to the inhibitory effect of cobicistat on creatinine secretion by the renal tubules. There was no change in median ratio of urinary protein to creatinine at week 48 compared with baseline. Median changes in bone mineral density (BMD) in spine and total body less head (were positive through week 48, and median changes in height-age adjusted spine and total body less head *z* scores were minimal. Only 1 participant experienced a decrease of higher than 4% in BMD in spine or total body less head at week 48 compared with baseline. These positive results favor the continuation of investigations of TAF, elvitegravir, cobicistat, and emtricitabine in the treatment of HIV-infected children and adolescents.

In Abstract 819, Giacomet and colleagues presented findings on the 10-year effects of TDF on BMD in 26 HIV-infected youths aged 4.9 years to 17.9 years in a longitudinal study. Eligibility criteria included HIV infection through MTCT,

undetectable viral load, and treatment with lamivudine, stavudine, and a PI. At enrollment, stavudine was changed to TDF and PIs were changed to efavirenz. Annual DXA scans were performed for 10 years. Lumbar spine and whole skeleton BMD values were compared with those of the control group, which consisted of 201 uninfected youths aged 3 years to 25 years. Regression curves of lumbar spine and whole skeleton BMD (absolute values and *z* scores) were statistically significantly lower in HIV-infected youths compared with controls, and the difference between HIV-infected youths and controls did not change over the 10-year period. The investigators concluded that switching to a TDF-containing regimen in HIV-infected youths was not associated with normalization of BMD values, yet the strategy did not lead to further decline in BMD over the long term.

Giaquinto and colleagues examined the pharmacokinetics, safety, and efficacy of maraviroc in addition to optimized background therapy in 103 R5-tropic HIV–infected, treatment-experienced children in an open-label, 2-stage trial (Abstract 818). Dose finding was assessed in stage 1, and safety and efficacy were evaluated in stage 2. The children were assigned to 1 of 4 age and formulation groups, with maraviroc dosed twice daily. At the start of the study, the maraviroc dose was based on body surface area and the optimized background therapy that the children were receiving, and the dose was adjusted and pharmacokinetics reevaluated if average concentration was below 100 ng/mL at week 2. Maraviroc was generally well tolerated, with most treatment-emergent adverse events being of grade 1 severity and no grade 3 or 4 treatment-emergent or serious adverse events being related to maraviroc. Fourteen children experienced grade 3 or 4 laboratory abnormalities, with the most common being grade 3 neutropenia. The safety profile of maraviroc in these children was considered to be comparable to that in adults. The children experienced a median decrease in HIV RNA of over 1 $\log_{10}$ copy/mL at week 48, with 48% of the children achieving an HIV RNA of less than 48 copies/mL. All children in the study had an increase in CD4+ cell count and percentage.

Optimizing the PMTCT Cascade

Gupta and colleagues presented more data on the roll-out of Malawi's Option B+ program from 2011 to 2015, with full decentralization of HIV treatment services and integration with PMTCT programs (Abstract 789). Using quarterly reports collected from all sites on cohorts receiving antiretroviral therapy, the investigators described the marked increase in the number of active sites offering antiretroviral therapy, from 303 to 704, and the rise in the percentage of antenatal clinics offering antiretroviral therapy, from 37% to 98% between 2012 and 2015. There was rapid integration of PMTCT and HIV treatment services, with 94% of sites providing services to those receiving antiretroviral therapy under Option B+ or in general between 2014 and 2015, and redistribution to smaller rural health facilities. Antiretroviral therapy coverage among HIV-infected pregnant women substantially increased from 22% to 95% between 2014 and 2015. There was a slight decrease in the proportion of sites with retention rates of higher than 80%, from 56% to 49% during the 4 years of the Option B+ program.

In Abstract 790, Aliyu and colleagues presented the results of a cluster randomized study to evaluate whether an intervention that offers a package of services improves maternal initiation of antiretroviral therapy and retention in care compared with standard of care in mother-infant pairs postpartum across 12 pair-matched sites in rural Nigeria. The intervention sites received the standard of care in addition to expanded service options, point-of-care CD4+ cell count testing, integrated maternal and infant services, and active involvement of male partners and peer mentors. The investigators found that the mothers who received the intervention were 3.3 times more likely to initiate treatment and 9 to 10 times more likely to be retained in care at 6 weeks and 12 weeks postpartum compared with women who received the standard of care.

In Abstract 791, Fayorsey and colleagues described the MIR4HEALTH (Maternal-Infant Retention for Health) trial, a randomized study examining the efficacy of a combination package of lay counselor–led interventions compared with standard of care to improve retention in PMTCT care in Kenya. Three hundred forty HIV-infected pregnant women initiating antenatal care were randomly assigned to 1 of 2 arms: 1) active patient follow-up, which involved individualized health education led by a lay counselor, phone and short message service (SMS) appointment reminders, home visits, physical tracing after missed visits, and support for retention and adherence (n = 170); or 2) standard of care, which involved routine PMTCT and postnatal HIV care according to Kenyan national guidelines (n = 170). The primary end point was attrition at 6 months postpartum, defined as the proportion of mother-infant pairs not retained in care as a result of loss to follow-up, pregnancy loss, or mother or infant death. Approximately one-third of the women were known to be HIV-infected, with a median (IQR) gestational age of 24 weeks (range, 17-28 weeks); median (IQR) CD4+ cell count was 426/µL (274/µL-601/µL). In intent-to-treat analysis, a statistically significantly lower attrition of mother-infant pairs was noted in the intervention arm than in the arm that recieved the standard of care (18.8% vs 28.2%; $P = .04$). Factors associated with lower attrition at 6 months postpartum were older age, known HIV infection at baseline, and disclosure of HIV serostatus to a partner. Nine infants tested positive for HIV infection on a PCR assay, with no statistically significant difference between the 2 arms. These findings highlight that a combination package of lay counselor–led interventions can result in a reduction in attrition in mother-infant pairs receiving PMTCT care in settings with high HIV prevalence.

Clouse and colleagues used an existing national laboratory database to ascertain the frequency of "clinic shopping" or switching of medical care postpartum among 312 women who had initiated antiretroviral therapy during pregnancy in 7 clinics in a South African province and were considered lost to follow-up (no documented clinic visit in more than 3

months) (Abstract 792). Women were categorized as having continued HIV care if they accessed care after initiating antiretroviral therapy at a new facility, as demonstrated by at least 1 HIV viral load or CD4+ cell count test on record in the database. Women were considered to be "clinic shoppers" if they received care at a new facility within the same region as the clinic in which they initiated antiretroviral therapy. Of the 284 women with available records who were considered lost to follow-up, a high proportion (37%) were actually in continued HIV care. Of these, 67% were clinic shoppers and 33% received care outside the province. The study findings emphasize the potential flaws in estimations of retention in care and highlight the importance of developing a national health database that can link patients and records by a unique identifier.

Retention rates among HIV-infected pregnant women enrolled in the Option B+ program in Malawi have ranged from 67% to 78%. Hoffman and colleagues conducted a case-control study of HIV-infected pregnant women who began antiretroviral therapy as part of Option B+, to examine sociodemographic characteristics, disclosure of HIV serostatus to partners, pre–antiretroviral therapy education, and knowledge regarding Option B+ among women retained in the program (Abstract 793). The study included 50 women who defaulted and were out of care from Option B+ for more than 60 days and 153 controls who were retained in Option B+ for at least 12 months. More than 80% of the women initiated antiretroviral therapy at a median (IQR) gestational age of 24 weeks (range, 16-28 weeks); of these, 91% defaulted and were out of care at 3 months postpartum. Women who were retained in care (controls) were more likely to disclose their HIV serostatus to their primary partner compared with those who defaulted (100% vs 78%; $P < .001$). In a multivariate analysis controlled for age, education, and travel time to the clinic, the odds of retention were higher among women who were aware of their partner's HIV serostatus (OR, 4.07; 95% CI, 1.51, 10.94) and had more knowledge about Option B+ (OR, 1.60; 95% CI, 1.15-2.23; $P = .004$). To enhance retention of HIV-infected pregnant women in Option B+ in Malawi, the investigators recommended interventions to help encourage disclosure of HIV serostatus to partners and improve education about the importance of the Option B+ program for maternal and child health.

Ebola Update

A special session was dedicated to the devastating Ebola outbreak in West Africa (2013-2016). According to the WHO, there have been more than 28,000 Ebola infections and 11,316 deaths from the Ebola virus, including 500 to 1000 health care workers.[19] There is a crucial need for vaccine development and treatment strategies to prevent or mitigate the severity of Ebola virus infections. Two presentations from the PREVAIL (Partnership for Research on Ebola Vaccines in Liberia) trial were dedicated to vaccines and immune-based therapy.

Bolay presented findings from a randomized controlled trial of the safety and immunogenicity of 2 Ebola vaccines in a phase II study of uninfected volunteers who were enrolled in the context of the Ebola outbreak in Liberia (Abstract 76LB). Uninfected volunteers were randomly assigned to receive 1 of 2 candidate vaccines or a placebo. The 2 candidate vaccines were a vesicular stomatitis virus (VSV)-based vaccine, rVSV-deltaG-ZEBOV GP, in which the gene encoding the G envelope glycoprotein of VSV is replaced with the envelope glycoprotein of the Ebola virus, and a recombinant chimpanzee adenovirus type-3–based vaccine, ChAd3-EBO-Z, which contains a DNA fragment insert that encodes the Ebola virus glycoprotein. There were 500 participants in each of the vaccine arms and 250 participants in each of the 2 associated placebo arms. Study end points included safety and immunogenicity. At baseline, 6.3% of study participants had antibodies to Ebola virus infection despite no prior knowledge of infection. The percentages of participants who were diagnosed with HIV infection and syphilis at baseline were 5.2% and 5.1%, respectively. Overall, attendance of follow-up visits exceeded 98%. Both vaccines were well tolerated, with only minor site reactions and transient decreases in neutrophils that did not persist beyond the first week of follow-up. Immunogenicity was measured by enzyme-linked immunosorbent assay (ELISA). Excluding those individuals who were antibody positive at baseline, an antibody response at month 1 was noted in more than 85% of participants in each of the vaccine arms and fewer than 10% of participants in the placebo arm ($P < .001$).

Davey presented results from the PREVAIL II trial, a randomized controlled trial of an investigational treatment that comprises 3 monoclonal antibodies (ZMapp™) for acute Ebola virus infection (Abstract 77LB). Having demonstrated promising results in nonhuman primate models, the treatment was introduced in this trial during the latter half of the Ebola virus epidemic in 2014 and 2015. Individuals with acute Ebola virus infection from sites in Liberia, Sierra Leone, Guinea, and the United States were randomly assigned 1:1 to 3 infusions of the treatment plus standard of care or to standard of care alone. The study was conducted over 10 months and was stopped due to extinction of the Ebola virus and subsequent decreased enrollment. Instead of including 100 participants in each arm as originally planned, 36 participants were enrolled in each arm. One participant in the control arm was lost to follow-up. There were 21 deaths overall, of which 13 occurred in the control arm (mortality of 37.1%) and 8 occurred in the arm that recieved treatment plus standard of care (22.2%). Although there was a trend toward superiority in the treatment arm, the posterior probability of superiority was only 91.2% and fell short of the predefined statistically significant threshold for declaring efficacy (set at ≥97.5%).

Little is known about the clinical sequelae after Ebola virus infection in survivors. Two presentations outlined long-term sequelae, the presence of asymptomatic infections, and characteristics of Ebola virus in semen (Abstracts 73LB and 74LB). Etard and colleagues described long-term clinical sequelae, psychosocial consequences, and viral clearance rates among a cohort of survivors in Guinea (Abstract 73LB).

Guinean adult and child Ebola survivors were enrolled (n = 475) in the survivor cohort for 2 years of follow-up. Several long-term symptoms were reported including muscle and joint aches, headache, fatigue, abdominal pain, anorexia, and ocular symptoms. The most severe findings in the cohort involved abnormal eye exam results in 19% of survivors; there were cases of uveitis, episcleritis, keratitis, and cataracts. Anemia (hemoglobin level < 10 g/dL) was the most common lab abnormality. Semen analysis from 107 men revealed a 6% positivity rate, and the viral RNA persisted in semen for 9 months in 1 individual. Additionally, depression was a prominent disorder among survivors (25% of men and 18% of women).

Similarly, Fallah and colleagues from the PREVAIL III trial in Liberia presented data on the natural history of Ebola virus disease among survivors (Abstract 74LB). The analysis from the ongoing PREVAIL III cohort study included follow-up of more than 1000 survivors of Ebola virus disease and more than 1000 close contacts. Common persisting symptoms and exam findings among survivors included ocular, musculoskeletal, and neurologic abnormalities. In particular, uveitis was a prominent finding. The issue of detection of persistent Ebola virus in semen was also examined in this study. Of 97 male survivors, 38% had viral RNA detected in the semen at least once, and in some men the viral RNA was detectable intermittently. The longest period of time between Ebola virus disease and continued Ebola virus detection in the semen was 18 months.

In an effort to better characterize the dynamics of Ebola virus clearance in semen, Sissoko and colleagues studied rates of Ebola virus clearance in a prospective cohort study of male Ebola survivors in Guinea (Abstract 75LB). Of 26 men included in the analysis, 73% were found to have Ebola virus in semen, detected by RNA PCR assay at the initial analysis, and the rate of viral clearance in semen was slow. Ebola virus was present in the semen of 1 participant at 334 days after initial disease onset. Using individual patient data, investigators modeled the likelihood of a positive result for Ebola RNA in semen and suggested that 50% would have detectable Ebola virus in semen at 108 days and 10% would have detectable virus in semen at 325 days. Additional studies are needed to understand the potential significance of persistent Ebola virus in semen in terms of transmission risk, so that proper counseling can be provided.

Although Ebola virus is most known for the severity of its clinical presentation and high mortality rates, several studies documented evidence of asymptomatic infection. Richardson and colleagues described evidence of asymptomatic Ebola infection that was found in the quarantined hot spot of Kono, Sierra Leone (Abstract 72LB). The performance characteristics of the ELISA tests to detect Ebola exposure and the estimated proportion of asymptomatic Ebola infection in the population were examined. Serum samples from 30 survivors and 132 uninfected controls who underwent testing for Ebola antiglycoprotein and antinucleoprotein were analyzed. Receiver operator curves were constructed, and the assays were found to be comparable. The Ebola antiglycoprotein ELISA, found to have 96.7% sensitivity and 97.7% specificity at a cutoff of 4700 U/μL, was selected for use during the second phase of the study. Volunteers who had not reported Ebola illness from quarantined households underwent Ebola antiglycoprotein testing. Of 207 volunteers, there were 12 seropositive individuals who had not had any symptoms. After combining known infections with asymptomatic infections, 25% of infections were found to be asymptomatic. The findings raised questions about appropriate screening for exposure and whether people with asymptomatic infections should be evaluated for clinical sequelae or ongoing risk for transmitting the virus.

Ebola virus disease has particularly impacted health care workers, resulting in fragmented local health care systems. Ndawinz and colleagues reported on the impact of the Ebola virus epidemic on HIV care in Sierra Leone (Abstract 910). During 2014, 360 health care workers were infected with Ebola virus, and over this same period, there was a substantial decline in the number of patients receiving antiretroviral therapy in Sierra Leone.

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