Volume **23** Issue **1** March/April **2015**

Topics in Antiviral Medicine™

A publication of the IAS–USA

Highlights of the 2015 Conference on Retroviruses and Opportunistic Infections CME

Topics in Antiviral Medicine™

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Topics in Antiviral Medicine™

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Current and previous issues of *Topics in Antiviral Medicine* (as well as *Topics in HIV Medicine*) are available online at www.iasusa.org/pub.

ISSN 2161-5861 (Print)
ISSN 2161-5853 (Online)

Printed in USA on acid-free paper

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Topics in Antiviral Medicine™

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Overview

The IAS–USA offers this state-of-the-art activity as part of a nationwide CME effort for physicians on the evolving challenges of managing HIV disease.

Learning Objectives

On completion of this activity, the learner will be able to describe important new data presented at the 2015 Conference on Retroviruses and Opportunistic Infections and the potential clinical implications for patient care in the areas of:

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

Intended Audience

This enduring material is designed for physicians and other health care practitioners who are experienced in the principles of complex chemotherapy (eg, antiretroviral therapy) and are actively treating or will be treating patients with HIV infection.

This activity is also relevant for other practitioners, including nurse practitioners, nurses, physician assistants, pharmacists, and others.

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Financial Affiliations: Drs Buchbinder, Havlir, and Liu have participated in research trials that have received provision of medicines from Gilead Sciences, Inc. Dr Luetkemeyer has received grants awarded to her institution from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Pfizer, Inc. Dr Wilkin has served as a consultant to GlaxoSmithKline/ViiV Healthcare, has received research support awarded to his institution from Gilead Sciences, Inc, Bristol-Myers Squibb, and GlaxoSmithKline/ViiV Healthcare, and has also received travel support from GlaxoSmithKline/ViiV Healthcare. His spouse is an employee of Johnson and Johnson. Dr Wyles has received grants awarded to his institution from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, and Merck & Co, Inc, and serves on an advisory board for Bristol-Myers Squibb. Drs Ances, Currier, Olender, Spudich, Stevenson, Taylor, and Wong have no relevant financial affiliations to disclose. Dr Richman has been a consultant to Gilead Sciences, Inc, Hera Therapeutics, and Monogram Biosciences, Inc. Dr Benson has served on data and safety monitoring boards for GlaxoSmithKline and ViiV Healthcare. She has received research grants awarded to her institution from Merck & Co, Inc, Gilead Sciences, Inc, Bristol-Myers Squibb, ViiV Healthcare, and AbbVie. Her spouse, Dr Robert Schooley, was awarded research grants, paid to his institution, from Boehringer Ingelheim Pharmaceuticals, Inc, and Bristol-Myers Squibb. His institution has received payment for consultative advice or data monitoring committee service from Bristol-Myers Squibb, GlobeImmune, Gilead Sciences, Inc, and Monogram Biosciences. He serves as a consultant to CytoDyn and has stock options from CytoDyn and GlobeImmune. Dr Hirsch has no relevant financial affiliations to disclose. Ms Jacobsen has no relevant financial affiliations to disclose.

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Improving the Management of HIV Disease®: Full-Day Courses

The annual, **full-day**, **advanced** CME courses continue to focus on cutting-edge, scientifically rigorous issues presented by leading experts in the field, providing the latest insights and data on the full spectrum of HIV- and AIDS-related treatment issues.

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Tuesday, March 10, 2015
Cobb Galleria Centre

San Francisco, California
Friday, March 20, 2015
Grand Hyatt San Francisco

New York, New York
Tuesday, March 31, 2015
New York Marriott Marquis

Los Angeles, California
Wednesday, April 29, 2015
The Westin Bonaventure

Washington, DC
Wednesday, May 13, 2015
Capital Hilton

Chicago, Illinois
Monday, May 18, 2015
Chicago Marriott Downtown
Magnificent Mile

Evolving Strategies in Hepatitis C Virus Management: Small-Group Workshops

Part of the IAS–USA focus on the management of HCV infection, the **half-day**, **small-group**, intensive CME workshops are presented by leading experts in the field. Attendance is limited to 35 practitioners, so early registration is encouraged.

Atlanta, Georgia
Monday, March 9, 2015
Cobb Galleria Centre

San Francisco, California
Thursday, March 19, 2015
Grand Hyatt San Francisco

Los Angles, California
Tuesday, April 28, 2015
The Westin Bonaventure

Washington, DC
Tuesday, May 12, 2015
Capital Hilton

Chicago, Illinois
Tuesday, May 19, 2015
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Review

CROI 2015: Basic Science Review

Mario Stevenson, PhD

The 2015 Conference on Retroviruses and Opportunistic Infections (CROI) represents a forum that encompasses all facets of research on HIV/AIDS and its complications. CROI is a valuable venue for scientific and public health researchers, clinicians, policy makers, and community representatives to be updated on the latest advances in their specific areas of interest and beyond. CROI 2015 continued to surprise. New insights into the viral reservoirs that persist in the face of antiretroviral therapy were prominently featured, as were therapeutic approaches aimed at curtailing and eliminating persistent viral reservoirs in HIV-infected individuals. Basic science is providing surrogates that could be valuable in how viral reservoirs are measured and, ultimately, in how to gauge if they are being effectively eliminated.

Keywords: CROI 2015, HIV, cure, viral reservoir, posttreatment control, tropism, reservoir elimination

Viral Reservoir Studies

Development of a strategy to cure HIV-1 infection and development of an efficacious vaccine are the most challenging undertakings in HIV/AIDS research, as was discussed at the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 22 to 26. Although antiretroviral therapy has profoundly improved, the prognosis for individuals living with HIV/AIDS—the prospect of lifelong antiretroviral therapy—is not ideal for a number of reasons. Emergence of resistant viruses limits the effectiveness of antiretroviral drugs, ongoing immune inflammation leads to collateral damage even in virally suppressed individuals, and maintaining adherence will continue to be challenging for HIV-1–infected individuals on long-term therapy. Adherence may be affected by drug toxicity, cost of medications, mental health issues, and substance use. For this reason, a coordinated effort to develop therapeutic approaches that can lead to a viral cure is needed.

HIV-1 has adopted strategies with which to achieve lifelong persistence in the human host. This is perhaps best illustrated in a recent study by Henrich and colleagues in which 2 HIV-1–infected individuals underwent allogeneic hematopoietic stem cell transplantation (HSCT) for hematologic cancers.[1] In contrast to those in the Berlin patient,[2] donor cells in the 2 individuals in this study expressed the full complement of viral coreceptor molecules. The objective was to determine whether a component of allogeneic HSCT could promote a sustained remission from antiretroviral therapy. HIV-1 DNA was undetectable in peripheral blood and rectal mucosa following allogeneic HSCT and provided justification for interrupting antiretroviral treatment. Plasma HIV-1 RNA and cell-associated HIV-1 DNA were undetectable in these 2 individuals until 12 weeks and 32 weeks, respectively, after cessation of antiretroviral therapy. Therefore, although allogeneic HSCT led to an extended period of antiretroviral therapy–free remission in 1 individual, the virus still rebounded, indicating the presence of long-lived tissue reservoirs of HIV-1. Despite this sobering result, there are renewed efforts to chart the viral reservoirs that sustain HIV-1 in the face of antiretroviral therapy.

Mechanisms of Reservoir Persistence

Most of the attention with regard to viral reservoir persistence has focused on the reservoir of resting memory CD4+ T cells harboring latent HIV-1. The true longevity of latently infected CD4+ T cells is unknown, but given the immunologic role of memory CD4+ T cells, to recognize antigens in adulthood that were first encountered in childhood, it is likely to extend over years. The true longevity of viral reservoirs is further complicated by the fact that some memory CD4+ T cells undergo homeostatic proliferation, during which integrated latent proviruses could be duplicated during formation of daughter cells at mitosis, as originally demonstrated by Chomont and colleagues.[3] Although it is possible that latent and replication-competent proviruses can be duplicated through the process of homeostatic proliferation, the majority of proviruses amplified through this process would be expected to be defective, non–replication-competent proviruses. A number of studies have revealed that the vast majority of proviruses of HIV-1–infected individuals harbors deletions and hyper mutations that render the genome nonfunctional. Therefore, the extent to which homeostatic proliferation maintains a reservoir of replication-competent proviruses is unknown.

As featured in several presentations at last year's CROI,[4] there is clear evidence for duplication of proviruses at mitosis, as evidenced by the presence of identical clonal proviruses in peripheral CD4+ T cells from HIV-1–infected individuals taking suppressive antiretroviral therapy. In his plenary presentation at CROI 2015 (session PL-2),

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Hughes summarized a comprehensive analysis of HIV integration sites in peripheral blood mononuclear cells (PBMCs) from HIV-infected individuals taking suppressive antiretroviral therapy (Abstract 21). Of 2500 integration sites, 40% were in clonally expanded cells. Indeed, in 1 HIV-infected individual, more than 50% of the proviruses had emerged from a single clone. Proviruses with identical integration sites can only be the result of mitotic duplication of proviruses during clonal cell expansion. Further, some integration sites could drive cell proliferation if those sites result in dysregulation of a gene that is involved in cell proliferation. In such a case, an increased frequency of integration sites within specific genes and an increased frequency of identical integration sites within that gene would be seen. Numerous independent integrations in the same integration site were observed in 2 introns of the MKL/myocardin-like 2 (MKL2) and BTB and CNC homology 1, basic leucine zipper transcription factor 2 (BACH2) genes, which are involved in cell growth regulation. Some integration sites were identical, further indicating clonal expansion of cells harboring proviruses at those integration sites. Given the potential for insertional activation of cell growth–regulating genes, it is surprising that cancers are not reported at a higher frequency in HIV-1–infected individuals. Although integration near growth regulating genes provides an opportunity for maintenance of proviruses, a central question is whether this provides a mechanism for the maintenance of functional proviruses in the viral reservoir.

Cell proliferation, as driven by viral integration site, promotes proviral duplication.

Studies of Activity of the Viral Reservoir

A major challenge is identifying latently infected cells in HIV-1–infected individuals. Currently, latency is considered to comprise resting memory CD4+ T cells harboring transcriptionally dormant proviruses. As such, other than by the presence of viral DNA, a latently infected cell would be indistinguishable from an uninfected cell. Several presentations focused on examining the biologic significance of cell-associated viral RNA that can be detected in individuals taking suppressive antiretroviral therapy. Simonetti and colleagues (Abstract 105) previously identified a clonally expanded population of HIV-1–infected cells that was responsible for persistent viremia in a patient with metastatic squamous cell carcinoma. Antemortem and postmortem analyses were conducted to determine the tissue origin of this clonal variant. One provirus from a highly amplified clone, which produced the majority of viral RNA in plasma, was found to be enriched in tumor tissues. Further, this provirus yielded virus in cultures ex vivo when cocultured with CD4+ T cells. Therefore, biologically competent proviruses can be amplified through clonal expansion.

Similarly, Wiegand and colleagues (Abstract 106) conducted genetic analysis of cell-associated RNA in aviremic individuals to gauge their relationship to proviral populations and persistent viremia. Two aviremic and 1 viremic individuals were assessed. The frequency of G-to-A hyper mutations was detected at similar levels to their proviral DNA populations, and RNA was also found to match a clonally expanded population in 1 individual. As yet, it is unclear whether cell-associated RNA is a result of spontaneous reactivation of latently infected cells or whether the viral reservoir constitutes cells undergoing continuous low-level viral transcription. Further, whether the cells can be a source of virus if antiretroviral therapy is interrupted remains to be determined.

In an attempt to gauge the biologic significance of cell-associated viral RNA, Etemad and colleagues (Abstract 110LB) performed a retrospective analysis of HIV-1–infected individuals from 5 AIDS Clinical Trials Group (ACTG) studies who were virologically suppressed on antiretroviral therapy and who underwent analytic treatment interruption (ATI). The timing of virus rebound was based on a confirmed HIV-1 plasma RNA level of greater than 200 copies/mL or a single HIV-1 RNA level of greater than 1000 copies/mL. Individuals initiating antiretroviral therapy during early or acute HIV-1 infection had lower levels of pre-ATI viral RNA than those treated during chronic infection. Levels of cell-associated RNA were associated with time to viral rebound after interrupting antiretroviral therapy. It remains to be determined whether time to rebound following ATI can serve as a surrogate for the size of the viral reservoir that persists in the face of antiretroviral therapy. According to current models, viral recrudescence occurs following stochastic reactivation of a small number of founder, latent viruses. Because of the stochastic nature of the reactivation, time to reinitiation of detectable viremia might be highly variable and, as such, not a reliable measure of the reservoir. However, a recently published study from Rothenberger and colleagues indicates that viral recrudescence is fueled by the simultaneous reactivation of founders in lymphoid tissue.[5] This suggests the viral reservoir is larger and more active than previously suspected.

Bull and colleagues (Abstract 107) presented studies aimed at identifying the source of low-level viremia that persists in individuals taking suppressive antiretroviral therapy—evidenced by HIV-1 RNA level between 40 copies/mL and 500 copies/mL after 1 year of suppressive antiretroviral therapy. Sequences in the viral envelope region (C2-V5) were correlated with the integration sites using an integration site looping assay.[6] Analysis of integration sites was able to determine whether proviruses were present in proliferating cells and if they had been duplicated through cell division. Proviruses with identical integration sites were observed in 6 of 8 subjects who exhibited low-level viremia at repeated study visits. Two of the 6 subjects exhibited proliferating clones that had envelope sequences identical to those in low-level viremia, and 3 of the 6 subjects had sequences of

low-level viremia that were not linked to proliferating PBMC sequences and had evidence of ongoing viral evolution, suggesting that viremia was the result of residual viral replication. This study further underscores observations that duplication of proviruses during cellular proliferation can maintain a population of functional proviruses that can also contribute to low-level viremia that persists in individuals taking suppressive antiretroviral therapy. Collectively, these studies suggest that in patients taking suppressive antiretroviral therapy, HIV-1–infected cells and –uninfected cells may be distinguishable by more than the presence of proviral DNA. If a component of the reservoir is transcriptionally active and some of those transcripts are translated, then it could be predicted that that component is amenable to clearance by immune-based strategies such as therapeutic vaccination. More studies are required to determine the longevity of the transcriptionally active viral reservoir and its modulation by host immune responses.

An active viral reservoir may persist in individuals taking suppressive antiretroviral therapy.

Immunologic and Virologic Surrogates of Posttreatment Control

Hurst and colleagues (Abstract 111LB) retrospectively analyzed samples from SPARTAC (Short Pulse Antiretroviral Therapy at HIV Seroconversion), a randomized study of primary HIV infection that incorporated a treatment interruption after 48 weeks of antiretroviral therapy. A battery of immunologic and virologic endpoints was assessed to determine if any of those markers could predict the extent of posttreatment control after treatment interruption. T-cell exhaustion markers, such as T-cell immunoglobulin domain– and mucin domain–containing molecule-3 (TIM-3) in CD8+ T cells and programmed cell death 1 (PD-1) TIM-3 and lymphocyte-activation gene 3 (LAG-3) in CD4+ T cells, were associated with time to rebound when measured pretherapy. However, other than total viral DNA, there did not appear to be any viral markers associated with time to rebound when measured at baseline or at treatment interruption.

Non-CD4+ T-Cell Reservoirs

A long-standing debate in the field is the contribution of cells other than CD4+ T lymphocytes to viral persistence, particularly in the face of suppressive antiretroviral therapy. Although there is ample experimental evidence that tissue macrophages support viral replication in the simian immunodeficiency virus (SIV)-macaque model and in viremic HIV-1–infected individuals, there is less evidence supporting a role for myeloid cells in aviremic individuals. One of the challenges to assessing the contribution of myeloid cells to viral persistence is the difficulty in sampling tissue macrophage populations of sufficient purity and quantity to determine their infection status. In addition, macrophages are a heterogeneous population of cells residing in various locations, including the liver, lung, bone marrow, spleen, lymph nodes, and gut. Therefore, infection status in 1 population may not reflect what is happening in other populations. Kandathil and colleagues (Abstract 380) examined whether liver macrophages, also known as Kupffer cells, were an HIV-1 reservoir in individuals taking suppressive antiretroviral therapy. Purified liver macrophages from 3 human donors were infected with an R5-tropic GFP reporter virus and culture supernatants assessed for the presence of viral RNA. In addition, liver macro-phages were purified from tissue explants taken from HIV-1–infected individuals who were viremic (n = 1) or aviremic (n = 2). Liver macrophages infected in vitro supported the production of infectious virus for up to 6 months. Liver macrophages from both individuals taking suppressive antiretroviral therapy released infectious variants that could transmit to reporter cells, as evidenced by the presence of proviral DNA in those reporter cells. Collectively, these data suggest low-level infection of liver macrophages in these individuals taking suppressive antiretroviral therapy, and additional studies are required to determine how generalizable this observation is. In addition, liver macrophages are difficult to access, therefore it will be important to evaluate whether more accessible tissue compartments, such as in the lungs and lymph nodes, harbor infected macrophages in individuals taking suppressive antiretroviral therapy.

Viral Tropism Studies

Another approach to assessing the contribution of macrophages to viral persistence is to examine the tropism of variants in plasma of HIV-1–infected individuals and those who undergo treatment interruption. Bednar and colleagues (Abstract 221) looked for evidence of macrophage tropism in viruses in the blood of HIV-1–infected individuals with late-stage infection. A primary determinant of macrophage tropism is the ability to use low levels of CD4. Previous studies from this group have demonstrated that macrophage-tropic viruses, gauged by the ability to use low levels of CD4 on the Affinofile cell line, are infrequent. The study determined whether macrophage-tropic viruses might be present in blood. Analysis of 18 subtype B and 20 subtype C, late-stage, HIV-1–infected individuals did not reveal examples of macrophage-tropic virus. However, some individuals harbored viruses that were more capable of infecting cells with low CD4 levels than typical R5 T-cell–tropic virus. That intermediate CD4 usage phenotype was previously seen in the cerebrospinal fluid (CSF) and in the genital tract. Further, this new group of intermediate viruses showed increased sensitivity to soluble CD4 that approximates what is seen with macrophage-tropic viruses. The investigators proposed that the appearance of intermediate phenotypes in the blood suggests some evolution toward macrophage tropism in compartments that are the origin of viruses in the blood in late-stage disease.

Rebounding viruses may originate from CSF in effectively virologically suppressed individuals.

In an extension of this analysis, the same investigators examined the CD4 usage of viruses that rebounded after treatment interruption (Abstract 112LB). Single genome amplification of the viral envelope gene was conducted to determine sequence diversity in the rebounding virus population. In addition, viral envelopes were cloned to examine tropism on the basis of CD4 usage. Phylogenetic analysis of viral envelope genes demonstrated that recrudescence of the viral population was from a small number of founder variants. Rebounding variants required high levels of CD4 for infection, and there was no evidence of macrophage-tropic virus. This analysis detected no myeloid cell source during viral recrudescence when antiretroviral therapy is interrupted. However, in this study, viruses were being sampled in blood, which does not exclude the possibility that viruses originating from tissue macrophages remain highly localized and tissue bound and do not enter the blood following treatment interruption. This was suggested in a presentation by Gianella and colleagues (Abstract 58). In that study, paired blood and CSF samples were collected from 14 chronically HIV-1–infected individuals taking suppressive antiretroviral therapy. At the earliest 2 time points after viral rebound, viral envelope and reverse transcriptase regions were amplified from cell-free HIV-1 RNA in blood and CSF. Ten of 14 participants demonstrated compartmentalization in viral sequences between blood and CSF in at least 1 gene. Seven participants exhibited very rapid recrudescence of viremia in CSF, suggesting that rebound originated within the central nervous system rather than viruses migrating from the periphery. This suggests that HIV reservoirs in the central nervous system contribute to viral rebound and that in the tissues, rebounding viruses may remain compartmentalized, thus escaping analysis when plasma is sampled.

Strategies for Reservoir Elimination

A number of presentations outlined ongoing efforts to eliminate long-lived reservoirs in individuals taking suppressive therapy. Because the reservoir of latently HIV-1–infected memory CD4+ T cells is considered the single biggest obstacle to viral eradication, investigators have been exploring "shock-and-kill" approaches designed to reactivate the virus from latency and render it susceptible to host immune responses or clearance by viral cytopathic effects on the host cell. Latency is considered to be primarily regulated at the level of transcription where proviruses in highly condensed regions of chromatin are not accessed by transcription factors that are necessary for efficient viral gene expression. Therefore, chromatin-modifying agents (eg, histone deacetylase [HDAC] inhibitors) relax chromatin and allow transcription factors to interact with proviruses and activate viral gene expression. Most of the focus to date has been on the impact of latency-reactivating agents on viral RNA levels. However, it will be necessary to reactive gene expression to the level at which viral proteins are being made if viral cytopathicity or host immune-mediating clearance are to take effect.

Several presentations examined the impact of Toll-like receptor 7 (TLR7) agonists in elimination of latently infected cells. Sloan and colleagues (Abstract 417) demonstrated that the investigational TLR7 agonist GS-9620 activated the virus ex vivo in PBMCs of HIV-1–infected individuals taking suppressive antiretroviral therapy. GS-9620 is a selective TLR7 agonist that is currently being evaluated in patients with chronic hepatitis B virus. In PBMCs from 11 of 12 individuals with undetectable HIV-1 RNA in plasma, GS-9620 activated viral RNA expression an average of 5.8-fold. (range, 2-fold to 26.8-fold across donors). The ability to induce HIV expression was reduced in subsequent treatments with GS-9620. In an extension of these observations, Whitney and colleagues (Abstract 108) examined the impact of a TLR7 agonist on plasma viremia in SIV_{mac251}-infected animals that were virologically suppressed on antiretroviral therapy. After 45 weeks of virologic suppression, macaques were given repeated doses of a TLR7 agonist at twice-monthly intervals while taking antiretroviral therapy. Cell-associated viral DNA was quantified in PBMCs and in colon and lymph node biopsies taken before and after completion of treatment with the TLR7 agonist. Although there was no obvious effect on plasma viremia after the first 3 doses of the TLR7 agonist, there were transient and consistent increases in plasma viremia (500 copies/mL-1000 copies/mL) between doses 4 and 7. Further, there were substantial reductions in viral DNA content in all tissue samples and a lower viral set point after cessation of antiretroviral therapy. These exciting findings pave the way for studies to assess the impact of TLR7 agonism on viral reservoirs in HIV-1–infected individuals taking effective antiretroviral therapy.

Financial affiliations in the past 12 months: Dr Stevenson has no relevant financial affiliations to disclose.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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Review

CROI 2015: Advances in HIV Testing and Prevention Strategies

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

HIV testing rates and awareness of HIV serostatus have improved globally, but disparities continue between black and white men who have sex with men (MSM) in the United States, and between women and men globally. The 2015 Conference on Retroviruses and Opportunistic Infections (CROI) was a watershed moment for preexposure prophylaxis (PrEP). Two efficacy trials conducted in MSM were stopped early because of an 86% reduction in the risk of HIV acquisition among men taking tenofovir and emtricitabine. New drugs, long-acting formulations, and different patterns of dosing are undergoing evaluation. Poor adherence has limited PrEP effectiveness in some populations, and new measures of drug levels in dried blood spots and hair appear to be promising new tools. Pharmacokinetic differences of PrEP agents in rectal versus vaginal tissue preclude extrapolating PrEP trial results among MSM to women. Several studies reported no HIV transmissions between HIV-serodiscordant couples when the seropositive partner was successfully treated for 6 months. However, consistent viral suppression does not occur in a substantial minority of patients in many clinics, reducing the potential impact of treatment as prevention.

Keywords: CROI 2015, epidemiology, HIV, injection drug use, phylogenetics, preexposure prophylaxis, PrEP, prevention, incidence, transmission, treatment as prevention, men who have sex with men, MSM, testing, serodiscordant couples

Tracking the Epidemic

Phylogenetic Studies

At the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 23 to 26, Fraser provided an overview of how phylogenetic studies can inform HIV prevention efforts (Abstract 127). His first point was that phylogenetics can definitively rule out transmission between pairs of viruses because of the distance of common ancestors, but cannot definitively prove that a specific person transmitted to another. He then described phylogenetic studies of more than 17,000 HIV-seropositive persons in the Netherlands, more than 10,000 of whom are men who have sex with men (MSM). He and his colleagues found that all of the 106 clusters detected continued through to the present day; none of them were eliminated, and 60% of the clusters in MSM originated before 1996.

One cluster accounts for 66% of the injection drug users (IDUs) studied. Although most clusters have similar transmission dynamics, making it unlikely that there is a core group responsible for transmission, the most recent clusters are those that are driving the epidemic, predominantly in younger persons. From these data, investigators estimate that approximately 70% of new HIV infections come from undiagnosed persons and that of this 70% at least 39% come from very early infection. Only 7% come from persons who have achieved viral suppression at least once, and the rest come from persons who have been diagnosed with HIV infection but are not virally suppressed. However, in response to a question after his talk, Fraser pointed out that the role of acute infection could be substantially less in epidemics with less concurrency of partners than in the epidemic among MSM in the Netherlands.

Marzel and colleagues also found that approximately 42% of new HIV infections in the Swiss HIV Cohort Study were due to recent infection (within the past year), and this number dropped to approximately 32% when recent infection was defined as occurring in the past 6 months (Abstract 244). They also found that the total viral load in the chronic phase of infection was negatively associated with the contribution of acute infection (ie, higher viral loads in the chronic phase were associated with a lesser contribution of acute infection to transmission).

Mehta and colleagues reported on the role of bridging individuals in HIV transmission in the San Diego Primary Infection Cohort (Abstract 246). In their analysis, persons with the greatest "uniqueness" scores were most likely to be central to networks, bridging dissimilar individuals. The investigators suggest that disassortative partnerships may disproportionately drive HIV epidemics.

Oster and colleagues presented data on clusters of genetically related viruses from 19 states in the United States (Abstract 241). They examined the growth of clusters from 2007 to 2012, a possible indication of ongoing transmission, and pointed to populations that may benefit from partner

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services and interventions that link newly diagnosed persons with HIV infection to care. They found that growth in the previous year (2006-2007) and the proportion of a cluster that were MSM statistically significantly predicted future cluster growth. Of note, race and ethnicity were not associated with cluster growth. Although most clusters were geographically limited, some clusters spread into other geographic regions, supporting the importance of collaboration between health department jurisdictions.

HIV Prevalence and Knowledge of HIV Serostatus

Espinoza and colleagues reported on differences in the rates of new HIV diagnoses in different metropolitan statistical areas (MSAs) in the United States from 2003 to 2012 (Abstract 1040). Overall rates of new HIV diagnoses in the 104 MSAs examined decreased by 3.7% per year. However, increases in rates of new diagnoses were seen in some MSAs among MSM, blacks, and whites. Diagnoses among MSM aged 13 years to 34 years increased or were stable, and rates were stable or decreased among MSM aged 35 years or older. These authors suggest targeted prevention planning within MSAs to address local epidemics and trends.

Wejnert and colleagues presented US Centers for Disease Control and Prevention (CDC) data from nationally representative surveys of MSM in 20 US cities (Abstract 1041). Black MSM were statistically significantly more likely to be HIV infected than white MSM among all age groups, with greatest disparities in the youngest age group; among MSM aged 18 to 24 years, 1 in 5 blacks was HIV seropositive compared with less than 1 in 20 whites and less than 1 in 13 Latinos. The disparities became even greater during 2008 to 2011, particularly for younger men. Knowledge of HIV serostatus was also considerably lower among black MSM than white MSM in all age categories younger than 50 years. Less than half of the black HIV-seropositive MSM younger than 30 years were aware of their serostatus, and less than two-thirds among all age categories were aware. However, black MSM reported statistically significantly lower rates of condomless anal sex than other racial or ethnic groups. These data continue to underscore the high rates of HIV prevalence and low rates of HIV serostatus awareness among young black MSM, and the need to consider factors other than individual risk when addressing this severe epidemic.

In the United States, HIV prevalence is higher among black MSM than among white MSM, and the disparities increased from 2008 to 2011, particularly among younger men.

Hall and colleagues presented data from the US National HIV Surveillance System on the proportion of new HIV diagnoses in 2012 that were considered late (ie, presented with a CD4+ count lower than 200 cells/μL or an opportunistic infection within 3 months of diagnosis) (Abstract 999). In 2012, 24% of all new HIV diagnoses were late, but there was considerable geographic diversity and none of the MSAs achieved a rate lower than 20%. Among men, the proportion of late diagnoses was statistically significantly higher among IDUs and heterosexuals than among MSM. Racial and ethnic disparities existed in many MSAs. Of 105 MSAs, late diagnoses were more common in blacks than whites in 38 MSAs, and late diagnoses were more common in Hispanics than whites in 68 MSAs. These data point to the ongoing challenge of late HIV diagnosis and the need for more widespread HIV testing.

Truong and colleagues presented data on HIV test–seeking behavior among blood donors in Sao Paulo, Brazil (Abstract 1010). Among nearly 12,000 donors, 55% had not heard of alternative public HIV testing sites and 2.7% reported that HIV testing was their primary reason for donating blood. Test seeking was associated with dissatisfaction with a prior alternative testing experience ($P = .004$) and hepatitis C virus infection ($P < .001$), suggesting that improvements in the availability and quality of alternative testing sites are needed to deter high-risk persons from donating blood during the window period for HIV detection.

Ellman and colleagues presented data on awareness of HIV diagnosis in the nationally representative Swaziland HIV Incidence Survey, comparing results from 2011 with results from 2007 (Abstract 1013). Of more than 18,000 men and women aged 18 to 49 years, 32% were HIV seropositive. Of these, 38% were unaware of their HIV infection. Fifty-five percent of HIV-infected individuals reported in a 2007 survey that they had never received an HIV test, compared with only 16% of individuals in a 2011 survey. After adjusting for marital status, education, and employment, men were statistically significantly more likely to be undiagnosed than women (adjusted odds ratio [aOR], 2.54; 95% confidence interval [CI], 2.25-2.87), and younger adults were more likely than older adults to be unaware of their HIV infection (aOR, 2.44; 95% CI, 2.04–2.86). These data point to the successful scale-up of HIV testing and the need for testing targeted toward men and younger adults, and for regular HIV screening in areas with high HIV seroprevalence.

Internationally, the proportion of persons who have never had an HIV test has decreased substantially. Men are consistently less likely to be aware of their HIV infection than women, and younger persons are more likely to be unaware in some settings.

Huerga and colleagues presented similar population-based data from KwaZulu-Natal, South Africa (Abstract 1014). Of nearly 6700 persons, 85% agreed to participate. HIV prevalence was 21% among women and 16% among men. In multivariate analysis, factors associated with lack of HIV testing and unawareness of HIV serostatus were younger age (<35 years), male sex, and having more than 1 sex partner in the prior 12 months. These data

show substantial improvement in the proportion of the general population that has received an HIV test, but also support the need for programs targeting men, younger adults, and persons with more than 1 sex partner.

Improving HIV Diagnosis, Including Acute Infection

Linley and colleagues presented data from 2008 to 2012 on the proportion of persons newly diagnosed with acute HIV infection from the US National HIV Surveillance System (Abstract 1043). They defined acute infection as having a documented or self-reported negative HIV antibody test result within 60 days of the first HIV antibody–positive specimen. Among more than 48,000 persons with a previous negative diagnostic HIV test result, 3.2% were diagnosed as having acute HIV infection. Acute HIV infection was statistically significantly more common among individuals aged 13 to 29 years, MSM, those with a documented recent negative HIV test result, inpatient and outpatient settings (compared with STI clinics or counseling and testing sites), and being located in the Northeast or West of the United States. Persons with acute HIV infection were also statistically significantly more likely to have a plasma HIV RNA level of greater than 1 million copies/mL.

Hoenigl and colleagues presented data on the risk factors associated with acute and early HIV infection among MSM in San Diego, California (Abstract 1021). In a cohort of nearly 9000 MSM, 200 were diagnosed with acute and early HIV infection. In multivariable analysis, the strongest risk factors for predicting acute and early HIV infection in this cohort included engaging in condomless receptive anal sex with an HIV-seropositive partner or with 5 or more partners, number of male sex partners, a syphilis diagnosis, and methamphetamine use. These behavioral risk factors help identify a population that should undergo frequent screening and should receive highly effective HIV prevention programs, such as preexposure prophylaxis (PrEP).

Strategies to Improve Uptake of HIV Testing

A number of investigators have attempted strategies to increase HIV testing in emergency departments (EDs) over the last several years. Two large studies of HIV testing in EDs were presented at CROI this year. Quinn presented data on temporal trends in HIV testing and knowledge of serostatus among patients who visited The Johns Hopkins University ED during the 25-year period from 1986 to 2013 (Abstract 98). The investigators performed identity-unlinked HIV and hepatitis C virus testing and chart abstraction over a 6- to 8-week period on all adult patients who had blood drawn for other reasons in 1987, 1988, 1992, 2001, 2003, 2007, and 2013. The testing was unlinked from patient identification information and was used to monitor trends in a variety of health outcomes. HIV prevalence peaked at 12% in 1993 and declined to 6% in 2013, and knowledge of HIV serostatus increased from 20% in 1987 to 93% in 2013.

Along with this improvement in knowledge of HIV serostatus was an increase in the percentage of individuals linked to care within 90 days of HIV diagnosis from 47% in 2005 to 88% in 2013. Antiretroviral therapy use also increased over this time period (from 27% in 2007 to 80% in 2013) as did viral suppression (from 22% in 2001 to 60% in 2013). Perhaps as a result of increased diagnosis and treatment of HIV-infected persons in the community, HIV incidence declined over this time period from a high of 2.5% per year in 2001 to a low of 0.2% per year in 2013, as measured by a multiassay algorithm used on cross-sectional samples. The investigators concluded that clinical HIV testing of ED patients increased from less than 1000 patients in 2005 to nearly 6800 in 2013, highlighting the value of reaching otherwise underserved populations through ED visits.

Conversely, Chavez and colleagues reported on the challenges of expanded HIV testing in the HIV Prevention Trials Network (HPTN) 065 study, also called the TLC-Plus (Test, Link to Care, Plus Treat) study (Abstract 1100). They worked with 9 hospitals in the Bronx, New York, and 7 in Washington, DC, encouraging them to increase HIV testing in EDs and inpatient units through a variety of methods, including increased staff and administrative support, simplified informed consent processes, electronic documentation of testing, and a switch from point-of-care testing with dedicated staff to laboratory-based testing. Investigators tracked data from more than 1.2 million ED visits and 360,000 hospitalizations in the Bronx and more than 700,000 ED visits and 150,000 hospitalizations in Washington, DC, from February 2011 to January 2014. Overall, only 6.5% of ED visits and 13% of hospitalizations included HIV testing in the Bronx, with no substantial increase over time. Similar results were seen in Washington, DC, with rates of 13.8% and 22%, respectively. There was also no net increase in the proportion of HIV tests with positive results across the sites. The investigators acknowledged the many logistical and staffing issues faced in these inner city hospital settings.

In another approach to medically based testing, Shaw and colleagues presented data from Canada on pre-diagnosis health care utilization patterns among patients testing positive for HIV (Abstract 1054). Their team found that antibiotics were more than 3 times more likely to be prescribed for HIV-infected persons in the year before their diagnosis than for persons in a control group. Although having a single prescription for antibiotics is not a specific marker of HIV risk—many HIV-uninfected patients were also prescribed antibiotics—dispensing antibiotics could serve as a reminder to practitioners to offer HIV testing in appropriate situations.

Several investigators have used home HIV self-testing as a strategy to reach persons who are not routinely tested in clinical settings. Medline and colleagues used a geosocial networking app to advertise the availability of free home HIV self-testing kits for MSM provided by mail, a voucher used

at a pharmacy, or via vending machine (Abstract 1098). Online advertising over a 6-week period in April and May of 2014 yielded nearly 12,000 unique visits to their website and 334 requests for HIV tests. Two-thirds of participants requested mailed tests and 30% requested pharmacy vouchers. Of 122 study participants reporting their behaviors and test results, 28% had been tested for HIV more than 1 year previously and 11% had never been tested. Two participants reported newly diagnosed HIV and both reported linkage to confirmatory testing or care. The investigators reported that this is a potential strategy for disseminating HIV testing to a population who do not seek regular HIV testing in a clinical setting.

Sabharwal and colleagues reported on the use of HIV home testing among newly diagnosed HIV-seropositive MSM in New York City (Abstract 1097). Compared with more than 2000 men who had not previously used home HIV tests, those who had used home HIV tests were significantly less likely to be black (23% vs 37%; $P = .007$) or Hispanic (23% vs 31%; $P = .007$). Home testers were significantly more likely to have had regular HIV testing (83% vs 62% tested in the past 12 months; $P = .002$). This suggests that home HIV testing is not reaching the populations most heavily impacted by HIV (those not routinely testing and persons of color). In response, the New York City Health Department plans to offer home HIV testing kits to partners of men newly diagnosed with HIV who decline testing in traditional settings. Although the investigators report that linkage to care within 3 months of HIV diagnosis was similar between home test users and nonusers (63% vs 69%), rates of linkage need improvement in both groups, and this study would not have been able to identify men with positive home HIV test results that do not present for confirmatory testing.

New HIV testing strategies are needed, such as finding novel ways to distribute home HIV self-tests and promoting HIV testing for partners of individuals diagnosed with sexually transmitted infections.

Golden and colleagues presented data from the 28 months before to the 28 months after the launch of an initiative for health departments to provide partner services for all partners of MSM newly diagnosed with bacterial sexually transmitted infections (STIs) in Washington state (Abstract 1099). The proportion of MSM who received partner services increased from 62% preintervention to 76% postintervention ($P < .001$), as did HIV testing among patients identified through partner services (from 63% to 91%; $P < .001$). The absolute number of new HIV diagnoses among patients diagnosed with an STI increased from 61 preintervention to 104 postintervention. The investigators conclude that making HIV testing an explicit component of partner services for STI treatment can increase HIV case finding.

Internationally, hybrid models of community health campaigns and home-based HIV testing appear successful.

In international settings, community health campaigns (CHCs) and home-based HIV testing have been demonstrated to reach large populations of previously untested or undiagnosed individuals. Chamie and colleagues presented data from the SEARCH (Sustainable East Africa Research of Community Health) trial, which used a hybrid model of both types of testing in 32 communities in eastern and southwestern Uganda and western Kenya (Abstract 1101). Investigators began with a 2-week, mobile CHC that tested individuals for various diseases (eg, diabetes, hypertension, malaria) including HIV. Individuals who did not respond to a CHC were offered HIV testing at home. Eighty-nine percent of all stable adult residents and 80% of all adult residents were reached through this model; 80% were reached through CHC and 20% through home-based testing. HIV prevalence was 9.4%, median CD4+ count was 516 cells/μL, and 43% of adults tested reported no prior HIV testing.

In a multivariate analysis, predictors of testing with home-based tests rather than through CHC included male sex, being unmarried, having HIV infection, having a nonfarming occupation, having a high education level, and spending more time away from the community in the year prior to the start of the study. Despite reaching more men through home-based testing, a sex disparity in testing remained at all ages, with 80% to 90% of stable men compared with 90% to 95% of stable women being tested for HIV in this hybrid model; rates of testing were lower among nonstable populations. This hybrid model reached large populations of untested and undiagnosed persons in these settings but highlights a continued need for strategies to reach men and mobile populations.

Risk Factors for HIV Transmission and Acquisition

MSM

Baral reported on the state of the HIV epidemic among MSM globally, with a focus on sub-Saharan Africa (Abstract 73). He pointed out that certain characteristics of the epidemic are similar globally: an HIV prevalence of 14% to 18% among MSM throughout most of the world and an increased risk among younger MSM. Surveys from many countries also suggest that 30% to 60% of MSM in sub-Saharan Africa have sex before age 18, pointing to the need to reach adolescent MSM. Baral also reported an HIV incidence rate of 5% to 20% per year throughout much of sub-Saharan Africa, including a consistent incidence rate of 7% to 10% in a single cohort in Kenya over many years. Bacterial STI rates among MSM are quite high (20%-30% each for syphilis and rectal gonorrhea), and these STIs are asymptomatic, therefore requiring regular STI screening. Across sub-Saharan Africa, 30% to 60% of MSM meet their sex partners online. In Lesotho and Swaziland, meeting same-sex sex partners online was associated with several measures of stigma

and an increased risk of HIV infection or depression. In Nigeria, after enactment of laws prohibiting same-sex marriage and criminalizing various aspects of MSM behavior, the proportion of MSM seeking health care declined, and other measures of stigma (eg, being blackmailed or verbally harassed) increased. Baral closed his talk with a plea to further evaluate the epidemiology of and risk factors for HIV acquisition among MSM in sub-Saharan Africa; to apply what is being learned about effective biomedical, behavioral, and structural interventions in other contexts to this one; and to frame the implementation science questions central to effectively addressing the MSM epidemic in sub-Saharan Africa.

Scott and colleagues presented data on a personalized, online risk-assessment tool for MSM called SexPro (Abstract 1017). Using data from 561 HIV seroconversions occurring among nearly 9000 MSM enrolled in 2 previous efficacy studies (EXPLORE and VAX004), investigators created an algorithm for predicting risk that they then applied to the 1164 HIV-seronegative black MSM enrolled in the observational HPTN 061 study. All 28 seroconversions in the HPTN 061 study were in the top 4 deciles of risk scores, as measured by SexPro. This suggests that SexPro may be useful in identifing MSM, including black MSM, at elevated risk for HIV infection and, therefore, a group to whom PrEP should be offered.

Youth

Santelli and colleagues reported on the marked increase in school attendance among girls and boys aged 15 to 19 years in the Rakai District of Uganda from 1994 to 2013 (Abstract 1036). School enrollment was associated with a lower likelihood of reporting ever having had sex (37% for those enrolled in school vs 88% for those not enrolled). Among sexually active women, those enrolled in school were less likely to have ever been pregnant (6% vs 78%, respectively), and to have HIV infection (2% vs 6%, respectively), and more likely to report consistent condom use (70% vs 17%, respectively). This suggests that strategies that increase school attendance could be useful in reducing HIV risk factors and incidence.

Injection Drug Use

Brady and colleagues reported on trends in HIV prevalence and risk among IDUs in Philadelphia, Pennsylvania (Abstract 1028). Philadelphia surveillance reported an 80% decline in new HIV diagnoses among IDUs from 2006 to 2013. Analyzing data from 3 National HIV Behavioral Surveillance (NHBS) cycles (2005, 2009, and 2012), the investigators found no overall trend in sharing of injection drug equipment. Multivariate analysis identified subgroups that were more likely to share injection equipment: IDUs who injected a combination of opiates and stimulants and those with less than a high school education (aOR, 1.43 and 1.56, respectively). Blacks and Latinos were less likely than whites to share injection equipment (aOR, 0.39 and 0.64, respectively). Persons using needle exchange programs were also less likely to report sharing injection equipment than those who obtained their injection equipment from dealers or varied sources (aOR, 0.61 and 0.51, respectively). Despite the tremendous strides made to decrease the number and rate of HIV infections in this population, needle exchange programs should be promoted to those not currently using these services.

Transactional Sex Among Women

Sionean and colleagues reported on the prevalence and correlates of transactional sex among heterosexual women in 21 US cities (Abstract 1031). The NHBS system interviewed 5507 women in 2010, of whom 19% reported receipt of money, drugs, or comparable items from a man in exchange for sex during the 12 months before the interview. HIV prevalence in the overall sample was 2.9% and was somewhat higher (4.5%) among women reporting exchange of sex, although this difference was not statistically significant. Characteristics and behaviors in the prior 12 months that were independently associated with exchange of sex in multivariate models (reported as adjusted prevalence ratio [aPR]) were structural (unemployment, aPR, 1.33; homelessness, aPR, 1.33; incarceration, aPR, 1.17), biomedical (STI diagnosis, aPR, 1.36), and individual behavioral (use of crack cocaine, aPR, 1.45). Women who exchanged sex were also more likely to have risky partners on multivariate analysis, including partners older by 10 years or more (aPR, 1.58), HIV-seropositive partners or those with an unknown serostatus (aPR, 1.20), partners who had been incarcerated (aPR, 1.25), and partners who were MSM (aPR, 1.16). This population of women with low socioeconomic status appears to be at greater risk than the general female population and needs access to STI and HIV prevention services.

Cowan gave a plenary presentation on the HIV epidemic among female sex workers globally (Abstract 135). The size of the global female sex worker population is unknown, but female sex workers are estimated to be 0.7% to 4.3% of the general population in sub-Saharan Africa, 0.2% to 2.6% of the population in Asia, and 0.2% to 7.4% of the population in Latin America and the Caribbean; rates are higher (≤9.1%) when transactional sex is included in the definition. A meta-analysis of the increased burden of HIV among sex workers found that compared with HIV prevalence in the general population, HIV prevalence among female sex workers is substantially higher: 12-fold higher in Latin America and the Caribbean, 12.4-fold higher in sub-Saharan Africa, and 29-fold higher in Asia. Modes of transmission studies, such as one conducted by the World Bank in Zimbabwe in 2012, suggest that a small proportion of epidemics originate in female sex workers (<5% in this example). However, when taking into account forward transmission of HIV, the population-attributable fraction can grow substantially over time. Structural interventions that reduce violence have been modeled to avert up to 20% of new infections among female sex workers, and decriminalization of sex work has

been modeled to reduce 33% to 46% of new infections. A meta-analysis of empowerment interventions for female sex workers suggests a reduction of new infections by one-third. Several studies of the care cascade in regard to female sex workers suggest that although progress has been made in some settings, additional interventions are needed to link women to care, provide antiretroviral therapy, and support ongoing retention in care. A cluster randomized trial that provides HIV-seronegative and -seropositive female sex workers with PrEP and antiretroviral therapy, respectively, is currently underway.

Zulu and colleagues reported on HIV incidence among adult patients in an STI clinic in Blantyre, Malawi (Abstract 1047). HIV incidence was 2.3 per 100 person-years, lower than the previous reported rate of 5.6 per 100 person-years. In multivariate analysis that included age, sex, marital status, income, and sexual risk practices, the only factor statistically significantly associated with HIV incidence was transactional sex (relative risk [RR], 2.4; 95% CI, 1.2-4.7).

STIs

Laga provided an overview of the impact of the HIV epidemic on global trends in STIs (Abstract 72). She pointed out that there was a substantial increase in the rate of STIs in the 1970s and early 1980s, variably attributed to the availability of oral contraception, changing cultural norms (eg, the sexual revolution, gay rights), urbanization, migration patterns, and increases in survival sex work. The response to the rising rates of STI incidence was primarily focused on improved diagnosis and treatment, with the emergence of antibiotic resistance, and placed little focus on primary prevention. With the early reports of AIDS and the recognition of widespread HIV infection, primary prevention became the focus, resulting in reductions in HIV and other STIs. However, since the rollout of antiretroviral therapy, a resurgence has been seen in the incidence of bacterial STIs such as syphilis, gonorrhea, and chlamydia. Laga pointed out that some HIV prevention interventions, such as male circumcision, may directly reduce STI acquisition (eg, herpes simplex virus 2 [HSV-2], human papillomavirus [HPV], bacterial vaginosis, chancroid). Other prevention strategies (eg, PrEP) will not directly reduce STI rates and must be paired with increased screening, diagnosis, and treatment, as well as searching for other primary prevention strategies.

Hormonal Contraception

Herold led a themed discussion session on the enduring controversy of the role of hormonal contraception in HIV acquisition risk (Session TD-S). She began the session by reviewing a meta-analysis that included 18 studies, more than 37,000 women, and 1830 incident infections.[1] In this analysis, compared with women not using any hormonal contraception, women who used depot medroxyprogesterone acetate (DMPA) were at statistically significantly increased risk for HIV infection (adjusted hazard ratio [aHR], 1.50; 95% CI, 1.251.83); women using norethisterone enanthate (NET-EN) or combination oral contraceptives were not at statistically significantly increased risk (aHR, 1.24 and 1.03, respectively). Neither age nor HSV-2 serostatus modified the effects of hormonal contraception on HIV acquisition. However, when the authors of the meta-analysis removed studies most likely to have been biased, the association between DMPA and HIV acquisition was attenuated (aHR, 1.22; 95% CI, 0.99-1.50).

Herold also described the various mechanisms by which progesterone could increase susceptibility to HIV infection, including thinning of the vaginal epithelium; disruption of tight junctions between epithelial cells; upregulation of syndecans; increase in proinflammatory cytokines or decrease in protective cytokines; increased CC chemokine receptor 5 (CCR5+) expression; changes in the microbiome; or increased susceptibility to other STIs which in turn could increase susceptibility to HIV. The authors of the meta-analysis point out that DMPA creates a more hypoestrogenic environment than either NET-EN or estrogen-containing combination oral contraceptives and that DMPA has a higher affinity for binding the glucocorticoid receptor than the progestins used in NET-EN or combination oral contraceptives. Either mechanism could potentially result in increased susceptibility to HIV, even when compared with other progestin-containing regimens.

Herold pointed out that much of the data to date have focused on studies in nonhuman primate models. At this year's CROI, Kersh and colleagues presented data on 16 pigtail macaques through several menstrual cycles and reported a statistically significant thinning of the stratum corneum of the vaginal epithelium (Abstract 862). In evaluating the 43 pigtail macaques studied in simian-human immunodeficiency virus (SHIV) challenge studies, investigators found a statistically significant correlation between the 4 days of the menstrual cycle that had the thinnest stratum corneum and the risk of SHIV acquisition.

Three posters presented in this themed discussion explored the role of hormonal contraception or phases in the menstrual cycle on expression of CCR5+, the coreceptor for HIV entry. Meditz and colleagues presented data on the effect of age and estrogen replacement following natural or medically induced menopause on CCR5+ expression in CD4+ cells in the peripheral blood (Abstract 859). They reported that after controlling for CCR5Δ32 genotype, older women had significantly increased CCR5+ CD4+ expression (4.2% increase with every 10-year increase in age; $P = .003$). Contrary to the investigators' hypothesis, CCR5+ expression did not increase after medically induced menopause, although estrogen replacement in post-menopausal women was associated with reduced CCR5+ expression in CD4+ cells.

Tsibiris and colleagues presented data from 92 women enrolled in the Women's Interagency HIV Study that had used DMPA, the levonorgestrel-releasing intrauterine device, or

combination oral contraceptives, and from 33 women who did not use hormonal contraception (Abstract 858). The investigators reported that CCR5+ expression in CD4+ and CD8+ T cells from the peripheral blood was higher among women using the levonorgestrel-releasing intrauterine device, with a nonstatistically significant increase in CCR5+ expression among women using DMPA. Haaland and colleagues presented data on changes in CCR5+ expression when moving from the follicular to the luteal phase of the menstrual cycle, when plasma progesterone increases (Abstract 860). They reported an increased proportion of memory T cells expressing CCR5+ and CD38+ during the transition to the luteal phase. When CD4+ T cells were stimulated, investigators found a significant increase in detectable intracellular tumor necrosis factor alpha (TNF-α; 31% vs 52%; $P = .006$) but no change in intracellular interleukin (IL)-2 or gamma interferon.

Roxby and colleagues presented data on changes to the vaginal microbiome in 15 HIV-seronegative Kenyan women initiating DMPA, followed up for a median of 8.5 months (Abstract 861). Gardnerella vaginalis, which was present in all the women before initiation of DMPA, declined by 0.21 $\log_{10}$ copies per swab per month ($P = .017$). DMPA initiation was also associated with significant declines in IL-6 ($P = .025$), IL-8 ($P = .041$), and the IL-1 receptor antagonist.

Grabowski and colleagues evaluated the association between DMPA use and HSV-2 risk in 682 HIV-seronegative and HSV-2–seronegative female partners of men enrolled in a circumcision trial in Rakai, Uganda (Abstract 28). Although 85% of the women used DMPA at some time during the trial, only 6% used DMPA consistently throughout the trial. Compared with women who were neither pregnant nor using hormonal contraception, consistent users of DMPA had an increased risk of HSV-2 acquisition (aHR, 2.26; 95% CI, 1.09-5.74). When the analysis was restricted to female partners of men with HSV-2 infection, the risk was even higher (aHR, 6.23; 95% CI, 1.49-26.3).

Stigma

Baugher and colleagues presented data on the prevalence and association of stigma with behavioral and clinical outcomes (Abstract 1057). Using data from the 2011 CDC-sponsored Medical Monitoring Project, an annual cross-sectional survey of a nationally representative sample of HIV-infected adults in the United States and Puerto Rico receiving HIV care, they reported that 76% of the 4385 participants endorsed 1 or more HIV-related stigma questions from a 6-item scale. Most commonly endorsed was the statement "It is difficult to tell people about my HIV infection," with 64% of the sample agreeing with this statement. Stigma scores were associated with depression, binge drinking in the past 30 days, nondisclosure of HIV serostatus to all sex partners, nonadherence to antiretroviral medications, and lack of viral suppression. The investigators emphasized the importance of developing and implementing stigma-reduction strategies to improve the health and quality of life of HIV-infected persons.

Balaji and colleagues presented data on the association between enacted stigma (verbal harassment, discrimination, and physical assault) and HIV-related risk behaviors among 8922 HIV-uninfected MSM recruited into the NHBS system in 2011 (Abstract 1058). Overall, 32% of the men surveyed reported verbal harassment within the past 12 months, 24% reported discrimination, and 8% reported physical assault. These 3 measures were associated with having 4 or more male sex partners, engaging in condomless anal sex, and engaging in exchange sex within the past 12 months. The investigators conclude that interventions that increase the acceptance of sexual minorities and help people to cope with stigma will be important for controlling the HIV epidemic.

Chan and colleagues reported on the association between antiretroviral therapy scale-up and reductions in reported stigma in the general populations of 18 sub-Saharan African countries (Abstract 1059). They reported that 89% of women and 81% of men aged 18 years to 49 years reported HIV-related stigma. However, investigators found that for each 1% increase in antiretroviral therapy coverage, there was a statistically significant decline in the percentage of women and men reporting HIV-related stigma. They note, however, that stigma prevalence was still quite high in all countries, and called for interventions to target HIV-related stigma.

Internationally, increased antiretroviral therapy coverage is associated with declines in reported HIV-related stigma.

Crowley presented an evaluation of HIV-related criminal law in the United States and Africa (Abstract 129). He pointed out that 61 countries, including the United States, have laws making it a crime to expose others to HIV, for HIV-infected persons to engage in certain sexual acts without disclosing their serostatus, and to transmit HIV to another person. Although Africa is the continent with the greatest number of countries with these laws, the United States is the country with the greatest number of prosecutions. Since 2008, there have been more than 200 HIV-related prosecutions in the United States. In all, 33 states have HIV-specific criminal laws, 11 states have laws making it a crime for HIV-infected persons to spit or bite, 29 states have had at least 1 prosecution in the last 2 years, and 10 states have punishments that include being made to register as a sex offender.

Crowley gave several examples, including that of an HIV-infected man in Michigan charged under the state's antiterrorism statute with possession of a "biological weapon"; that of an HIV-infected man in Iowa who was sentenced to 25 years after a single sexual encounter, even though a condom was used and the man had an undetectable viral load; and that of an HIV-infected man who is currently serving a 35-year prison sentence for spitting at a police officer. Crowley pointed out that many of these laws run counter to scientific estimates of transmission risk and that

data suggest these laws do not deter risky behavior and may, paradoxically, discourage persons from HIV testing and treatment.

In Africa, given the generalized epidemic, laws criminalizing HIV may affect a large proportion of the general population. In addition, homosexuality is illegal in almost all African countries, and MSM have disproportionately higher HIV infection rates than the general population. These anti-gay laws and policies may drive MSM away from needed services and thus contribute to rather than address local HIV epidemics. Crowley ended his presentation with a call to action for practitioners, asking them to seek out and understand local laws, find opportunities to present objective scientific evidence about HIV transmission risk, and to serve as role models for treating heavily impacted populations with respect, while explaining to colleagues why this is so important.

Stigma continues to drive the HIV epidemic and is associated with depression, substance use, nondisclosure of HIV serostatus to all sex partners, nonadherence to antiretroviral medication, and lack of viral suppression.

Smith and colleagues presented data on the incidence and risk factors associated with sexual assault among MSM and young women in coastal Kenya from longitudinal cohort studies since 2005 (Abstract 102). They reported that among 971 men (75% of whom were MSM) and 455 women (81% of whom were sex workers), the incidence of rape was 3.7 per 100 person-years and 4.5 per 100 person-years, respectively. Factors associated with rape among MSM included younger age, engaging in exchange sex, having only male sex partners, and engaging in group sex. For women, risk factors for rape included being HIV seronegative. Among MSM, HIV incidence was 11.7 per 100 person-years in the group who reported being raped, and 7.0 per 100 person-years in those who did not report being raped, a difference that was not statistically significant. These data indicate that rape is common among MSM and female sex workers, and that interventions are needed to reduce sexual, physical, and verbal abuse in vulnerable populations.

Population Mobility

Palk and Blower presented data on the relationship of mobility to risk practices and HIV infection within populations in Lesotho (Abstract 1034). Using data from the 2009 Demographic and Health Survey of Lesotho, they estimated that 30% of women and 32% of men traveled 1 to 4 times in the previous year, and 18% of women and 21% of men traveled 5 or more times. Men and women who traveled more were statistically significantly more likely to have more than 1 sex partner and were more likely to have concurrent sex partners. Men who traveled 5 or more times per year had a 30% higher risk of HIV infection than men who did not travel.

Prevention Strategies

PrEP

PrEP was featured prominently at this year's CROI. Landovitz's plenary session provided an update on the state of the science of PrEP and highlighted key implementation considerations (Abstract 20). PrEP involves administering antiretroviral medications to HIV-uninfected, at-risk individuals to lower their risk of HIV acquisition. Currently, the only PrEP regimen approved by the US Food and Drug Administration (FDA) is the fixed-dose combination of daily, oral tenofovir disoproxil fumarate (TDF) and emtricitabine. Several clinical trials have demonstrated the efficacy of TDF alone or in combination with emtricitabine among MSM and transgender women,[2] and among HIV-serodiscordant heterosexual couples and young heterosexual men and women in Africa.[3,4] However, a lack of efficacy was observed in 2 PrEP trials in African women,[5,6] owing in large part to low adherence rates in these studies. Nonhuman primate models have demonstrated lower tenofovir concentrations in the cervicovaginal than in the rectal compartment with oral TDF dosing, a finding also confirmed by a single-dose pharmacokinetic study in women.[7] These results suggest that there may be less forgiveness for nonadherence to TDF-based PrEP in protection against vaginal exposures. Landovitz pointed to a pharmacokinetic modeling study that suggested that 6 to 7 doses per week of TDF and emtricitabine is likely required to achieve high levels of vaginal protection against HIV in women.[8]

Although PrEP with daily, oral TDF and emtricitabine was approved by the US FDA in July 2012, adoption of this prevention strategy has been slow. Landovitz highlighted a number of concerns that have been raised regarding PrEP implementation and reviewed available data addressing these issues. One concern is risk compensation, or an increase in risk behaviors as a result of using PrEP, which could lead to a heightened risk of HIV acquisition at the individual and population levels. A modeling study of PrEP in resource-limited settings indicated that although risk compensation had minimal impact on population-level incidence at high levels of PrEP effectiveness, cumulative HIV infections could increase with risk compensation at lower levels of PrEP effectiveness.[9] Although risk compensation has not been observed in placebo-controlled PrEP trials, Landovitz affirmed the importance of monitoring for changes in risk behavior in upcoming PrEP implementation programs in which placebos will no longer be used. He noted an increase in rates of sex with nonprimary partners, observed after unblinding and release of efficacy results in the Partners PrEP study.[10]

Another concern that was raised is the emergence of HIV resistance among individuals who acquire HIV infection while taking PrEP. Modeling studies have yielded conflicting predictions, ranging from doubling of rates of transmitted resistance,[11] to a more limited impact on circulating resistance.[12] HIV resistance has been rare in

clinical trials of PrEP, observed in only 5 of 243 (2.0%) individuals who were randomly assigned to an active drug and became HIV-infected after enrollment, and only 0.06% of all those randomly assigned to active study drug. However, resistance with oral PrEP was more common when PrEP was initiated in the setting of undiagnosed primary HIV infection, with resistance seen in 8 of 29 (28%) participants.

HIV resistance appears to be rare with PrEP use and occurs most often in the setting of unrecognized acute HIV infection at the time of PrEP initiation.

A key clinical question is the timing of protection onset after PrEP initiation, and how quickly protection wanes after PrEP is discontinued. Based on data from an intensive pharmacokinetic study of intracellular tenofovir concentrations in peripheral blood mononuclear cells and a pharmacodynamic model derived from the iPrEx (Chemoprophylaxis for HIV Prevention in Men) study, it has been inferred that high levels of protection can be achieved after 5 daily doses of TDF and emtricitabine, and that a greater than 90% risk reduction persists up to 7 days after stopping treatment. Landovitz noted that these estimates are based on daily dosing and pertain only to MSM, as estimates may differ for other populations or routes of exposure.

Landovitz also summarized data on the safety of PrEP with TDF and emtricitabine when used in HIV-uninfected individuals across several clinical trials. First, a start-up syndrome with gastrointestinal symptoms has been observed in up to 18% of participants, although this appears to be self-limited and did not result in substantial PrEP discontinuations. Although TDF has been associated with renal toxicity in HIV-infected individuals, only 0.2% of participants randomly assigned to receive TDF and emtricitabine across PrEP studies experienced creatinine elevations of grade 2 or higher. TDF and emtricitabine has been associated with modest (0.4% to 1.5%) bone mineral density loss in HIV-uninfected individuals but not increased fracture risk, and bone mineral density appears to return toward baseline after drug withdrawal. As low adherence in PrEP trials may result in an underestimation of adverse events, Landovitz highlighted the need for longer-term safety monitoring and follow-up in diverse populations.

PrEP adherence is crucial for effectiveness, and adherence support is an important component of PrEP delivery. Several support strategies are currently being evaluated in PrEP demonstration projects, including counseling approaches, text messaging support, and use of electronic "smart" devices to provide real-time monitoring and intervention in the event of missed doses. Real-time drug-level testing is also being used in a PrEP demonstration project in Los Angeles, California, to triage participants with low drug levels to a staged adherence intervention.

Landovitz highlighted several studies which have indicated that cost-effectiveness is optimized when PrEP is targeted to individuals at highest risk for HIV acquisition. He pointed to an analysis of iPrEx data demonstrating that the impact of PrEP in MSM and transgender women is maximized when PrEP is targeted to those who reported engaging in condomless receptive anal sex, having a recent STI, or using cocaine in the past month.[13] He also provided an example of the importance of PrEP adherence in those at highest risk, by contrasting the iPrEx study, in which drug detection was higher in those reporting higher risk and which demonstrated a 42% overall effectiveness, with the VOICE (Vaginal and Oral Interventions to Control the Epidemic) study, in which drug detection was lower in those at higher risk and in which no overall effectiveness was seen. Early open-label PrEP studies have shown substantial uptake of PrEP, with greater uptake and adherence observed in higher risk populations in the iPrEx OLE (iPrEx Open-Label Extension).[14] Early data from the PrEP Demonstration Project in 3 US cities also show high levels of PrEP uptake and adherence.[15,16]

Adherence to PrEP is crucial for effectiveness. Novel strategies to measure and support PrEP adherence are being investigated.

There has been debate about the optimal setting for PrEP delivery. Landovitz referred to the "purview paradox" described by Krakower and colleagues: HIV practitioners believe primary care practitioners are best suited to prescribe PrEP because of their access to HIV-uninfected populations, and primary care practitioners do not feel they have the expertise to prescribe PrEP and monitor PrEP patients.[17] Progress is being made to build infrastructure for PrEP delivery, through leadership from community-based organizations, local health jurisdictions, consumers, and practitioners. Online lists of PrEP practitioners and resources are being developed, the national PrEP Warmline has been established, and methods for academic detailing are being implemented to expand practitioner capacity to deliver PrEP. Coverage for PrEP medications and services remains a challenge for some consumers who may face substantial out-of-pocket costs, although a number of programs are being established to address PrEP access issues, including the use of PrEP navigators and state programs to assist with the cost of PrEP medications or services.

Although PrEP with TDF and emtricitabine is a promising, approved prevention strategy, new PrEP drugs and formulations are being evaluated. The entry inhibitor maraviroc is an alternative oral medication being evaluated in the phase II HPTN 069 study. Two long-acting, injectable, investigational PrEP drugs, rilpivirine (formerly TMC278) and cabotegravir (formerly GSK744), are also being evaluated in phase II trials. Implementation of PrEP with TDF and emtricitabine is being evaluated in key at-risk populations, including black MSM and youth, 2 populations at particularly high risk for HIV acquisition.

Landovitz closed the plenary session with a call to combat the stigma faced by individuals seeking to protect themselves from acquiring HIV by using PrEP, recalling the damage of historical efforts to shame individuals seeking syphilis treatment and women seeking contraceptive services. He stressed the importance of supporting research and advocacy for individuals who are proactively seeking PrEP and the importance of "being on the correct side of history."

PrEP in Clinical Settings

Several investigators reported on the impact of PrEP in real-world clinical settings. McCormack and colleagues presented data from the PROUD (Pre-exposure Option for Reducing HIV in the UK) study, which enrolled 545 MSM from 13 sexual health clinics in England (Abstract 22LB). Participants were randomly assigned to receive daily TDF and emtricitabine either immediately or 12 months after enrollment. Based on early demonstration of efficacy, in October 2014 the Independent Data and Safety Monitoring Committee recommended that all MSM in the deferred-treatment arm be offered PrEP. HIV incidence was high in this cohort, with 19 new infections observed in the deferred arm (8.9/100 person-years), despite 174 prescriptions for postexposure prophylaxis (PEP) provided in this group. There were 3 incident infections in the immediate-treatment arm (HIV incidence 1.3/100 person-years), resulting in 86% PrEP efficacy (95% CI, 62%-96%; $P = .0002$) and a number needed to treat of 13 per year to avert 1 HIV infection. STIs were common but did not differ between the groups (57% vs 50% in the immediate- vs deferred-treatment arms, respectively; $P = .08$), and reported risk behaviors remained stable during follow-up (median number of anal sex partners was 10 at baseline and similar at month 12).

Baeten and colleagues presented interim data from the Partners Demonstration Project, an open-label prospective study delivering PrEP and antiretroviral therapy to heterosexual HIV-serodiscordant couples in Kenya and Uganda (Abstract 24). Antiretroviral therapy–naive, high-risk couples (n = 1013) were identified using a validated risk-scoring tool;[18] antiretroviral therapy was offered to all HIV-infected partners according to local treatment guidelines, and PrEP was offered as a bridge to antiretroviral therapy (eg, until the HIV-infected partner had been taking antiretroviral therapy for 6 months). Uptake of PrEP and antiretroviral therapy were high in the cohort (> 95% and approximately 80%, respectively). During 858 person-years of follow-up, PrEP was used during 48% of the follow-up period, antiretroviral therapy was used during 16%, a combination of the 2 was used during 27%, and nothing was used during 9%.

Only 2 incident HIV infections have been observed to date (HIV incidence 0.2/100 person-years) compared with 39.7 HIV infections expected (incidence 5.2/100 person-years) based on a counterfactual simulation model using data from a prior prospective study of HIV-serodiscordant couples, resulting in a 96% reduction in HIV transmission. The 2 transmissions occurred in the setting of low PrEP adherence. Heffron and colleagues presented data on PrEP discontinuations and adherence in the same study (Abstract 969). Among 985 HIV-uninfected partners who started PrEP, 314 (32%) subsequently had an HIV-infected partner who used antiretroviral therapy for at least 6 months, the majority (77%) of whom decided to stop PrEP because of sustained antiretroviral therapy use by their partner. Among a random sample of 133 HIV-uninfected participants using PrEP, plasma tenofovir levels were detected in more than 80% of samples tested during all periods when they were expected to have been using PrEP through month 12, suggesting that PrEP use is sustained among people with ongoing risk.

Glidden and colleagues presented data on PrEP engagement in iPrEx OLE (Abstract 970). Among 1603 HIV-seronegative participants enrolled in iPrEx OLE from prior PrEP trials, 1225 (76%) elected to initiate PrEP. At 1-month follow-up, less than half of the participants overall had a dried blood spot (DBS) level consistent with taking at least 4 pills per week, which varied substantially by site. In a multivariable model, age, education, number of partners, and engaging in condomless anal sex were associated with higher drug levels; cocaine or alcohol use and earning any income in the prior month were negatively associated with PrEP adherence (all $P < .05$). Gastrointestinal symptoms were associated with lower drug levels and explained approximately 7% of the variation in drug levels. Participants who missed their first follow-up visit or had drug levels indicating fewer than 2 pills per week were very unlikely to achieve higher drug levels at future visits. At month 12, only 38% of those who initiated PrEP had drug levels consistent with taking at least 4 doses per week. These results highlight the numerous challenges across the PrEP prevention cascade, which may require addressing factors at the individual and structural level.

Real-world studies of PrEP have demonstrated high levels of its effectiveness in the prevention of HIV acquisition among MSM and HIV-serodiscordant couples.

The Bangkok Tenofovir Study previously demonstrated that daily oral tenofovir reduced the risk of HIV acquisition by 49% among IDUs. Martin and colleagues presented early data from an open-label extension of this cohort offered 1 year of PrEP with tenofovir (Abstract 971). Out of 1327 IDUs who returned to receive trial results, 59% elected to take tenofovir, with most (84%) receiving doses in clinics. Based on adherence diaries, 78% of participants missed more than 8 doses in the past month, suggesting the need for additional adherence support in this cohort.

Mayer and colleagues provided evidence of increasing PrEP utilization at Fenway Health, a community health center in Boston, Massachusetts, specializing in sexual- and gender-minority primary care (Abstract

972). Based on a review of electronic medical records, PrEP prescriptions increased from 6 in 2011 to 326 in 2014 ($P < .05$ for upward trend), and PrEP users became more ethnically and racially diverse over time. PrEP was being provided by more than 40 practitioners at the health center, and more than 80% of prescriptions were covered by commercial insurance. A substantial proportion of patients were prescribed PrEP after a recent STI or after having used PEP, suggesting high-risk behaviors among PrEP users.

On a less encouraging note, Kelley and colleagues identified numerous barriers to achieving protection from HIV acquisition with PrEP among MSM in the US South (Abstract 973). Investigators proposed a PrEP care continuum similar to the HIV care continuum and projected the proportion of MSM who would reach each stage, based on data from the InvolveMENt study, an HIV incidence cohort of black and white MSM in Atlanta, Georgia. Among 562 participants, 50% were estimated to be aware of and willing to take PrEP, 65% had health insurance (20% were eligible for the Affordable Care Act), 69% of the cohort met eligibility criteria for PrEP, and 51% were estimated to be adherent to PrEP, based on data from the iPrEx study. Integrating these parameters into the PrEP care continuum

PrEP prescribing by practitioners is limited, and additional support to increase PrEP prescribing practices is needed.

resulted in only 15% of the overall cohort achieving theoretical protection from HIV acquisition (12% of black MSM and 18% of white MSM). The investigators conclude that disparities at various steps in the PrEP care continuum, particularly in access to health care, could lead to racial disparities in those achieving protection via PrEP; highlight the need for novel strategies for PrEP delivery to at-risk MSM; and point to the need for specific interventions to address barriers at each step of the PrEP care continuum.

Garg and colleagues presented data on PrEP prescribing practices among practitioners surveyed through the Medical Monitoring Project between January 2013 and January 2014 (Abstract 974). Among 1234 HIV practitioners who also cared for HIV-uninfected patients, an estimated 26% reported ever prescribing PrEP. Practitioners who were gay, lesbian, or bisexual were more likely to prescribe PrEP, as were male practitioners and those providing continuity care to more than 50 HIV-infected patients. Approximately 74% of practitioners prescribed PrEP to MSM, 30% prescribed PrEP to women who have sex with men, 23% prescribed PrEP to men who have sex with women, and 23% prescribed PrEP to HIV-serodiscordant couples. The researchers call for targeted efforts to increase PrEP prescribing, particularly among those practitioners who have limited experience prescribing antiretroviral therapy.

Nondaily PrEP Regimens

Several researchers presented data on the use of nondaily PrEP regimens for HIV prevention. Molina and colleagues reported results from the IPERGAY study, a randomized, placebo-controlled trial of pericoital PrEP among MSM in France and Canada (Abstract 23LB). Four hundred fourteen participants were enrolled and instructed to take 2 doses of TDF and emtricitabine or a placebo 2 hours to 24 hours before having sex, then 1 additional dose each at 24 hours and 48 hours, respectively, after the first drug intake. In October 2014, based on high levels of efficacy, the Data and Safety Monitoring Board recommended discontinuation of the placebo arm and offering of pericoital PrEP to all participants. There were 14 new HIV infections in the placebo arm (HIV incidence 6.6/100 person-years) and 2 infections in the arm receiving TDF and emtricitabine (incidence 0.94/100 person-years), indicating an 86% PrEP efficacy (95% CI, 40%-99%; $P = .002$) and a number needed to treat of 18. The 2 HIV infections occurred in individuals who had discontinued PrEP several months prior to infection and did not have detectable tenofovir levels in plasma at the time of seroconversion. The median number of pills used per month was 16, an average of 4 pills per week. Given data suggesting that 4 pills per week may yield efficacy levels similar to daily dosing among MSM, additional data are needed to determine how patterns of pill taking are related to sexual risk. Rates of most adverse events were similar across the study arms, with drug-related gastrointestinal events reported more frequently in the TDF and emtricitabine arm (13% vs 6%; $P = .013$). Sexual behaviors remained stable during follow-up though STIs were common, with more than one-third of participants diagnosed with at least 1 STI during the study.

Pericoital PrEP (before and after sex) was effective in MSM, with participants using 4 doses per week on average. More data is needed on the effectiveness of PrEP in the setting of less frequent sexual activity and dosing.

Bekker and colleagues presented results from the HPTN 067 study, also called the ADAPT study, of South African women in Cape Town (Abstract 978LB). Of 191 participants enrolled, 179 were randomly assigned to daily, time-driven (twice weekly with a postintercourse boost) or event-driven (before and after intercourse) dosing. Although fewer pills were required for sexual event coverage in the time- and event-driven arms, coverage of sex acts was higher in the daily arm (75%) than in time-driven (58%) and event-driven (52%) arms ($P < .001$). Adherence to the assigned regimen was also higher in the daily group than in the time- and event-driven groups, and adherence to the postsex dose in the nondaily arms was low. The majority of women had detectable tenofovir levels when sex within the past week was reported, with higher detection rates in the daily arm. Adverse effects were uncommon in the daily arm and were less frequent in the nondaily arms. The researchers concluded that daily dosing may foster better establishment of a pill-taking routine and provide the

most forgiveness for missed doses, supporting the current recommendations for daily dosing of PrEP with TDF and emtricitabine for women.

In a study comparing intermittent and daily PrEP in South African women, coverage of sex acts was highest in the daily-PrEP arm.

Rees and colleagues reported results from the Follow-on African Consortium for Tenofovir Studies (FACTS) 001 study, a phase III trial of pericoital 1% tenofovir gel for HIV prevention in South African women (Abstract 26LB). Overall, 2059 women were enrolled in the study and randomly assigned to receive 1% tenofovir or placebo gel using the BAT-24 (gel use within 12 hours before and after sex, with no more than 2 doses in 24 hours) dosing regimen; this same regimen was used in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 study, which demonstrated a 39% reduction in HIV incidence.[19] Enrolled women were young (mean age, 23 years), mostly unmarried, and the majority lived with parents or siblings. Overall, 123 women became HIV-infected postenrollment, resulting in an overall annual HIV incidence of 4.0 per 100 person-years; 61 HIV infections occurred in the tenofovir arm and 62 occurred in the placebo arm, yielding an incidence rate ratio of 1.0 (95% CI, 0.7-1.4). Based on returned applicator counts, women used the product on average for 50% to 60% of sex acts, a finding consistent with tenofovir drug level detection rates by cervicovaginal lavage. In a case-cohort analysis, having detectable tenofovir levels was associated with a reduction in HIV acquisition rates (HR, 0.52; 95% CI, 0.23-0.97; $P = .04$). Based on the study's findings, Rees highlighted the need for new prevention technologies that would be easier for young women to integrate into their lives.

A large confirmatory trial of pericoital 1% tenofovir gel in South African women showed no evidence of efficacy.

PrEP Pharmacokinetics and Measurement

Several presentations focused on the clinical pharmacology of HIV prevention and the use of pharmacokinetic measures to monitor adherence to PrEP. In a scientific overview presented by Boffito (Abstract 80), important pharmacologic properties of various PrEP agents were highlighted, including the ability to rapidly reach and accumulate in genital and rectal tissues, drug persistence in tissues, and protein binding, which may affect ability to penetrate mucosal tissues. For oral tenofovir, Boffito reviewed data showing that the tissue-to–blood plasma ratio was substantially lower in the cervix and vagina in women than in the rectum in men.[7] Similarly, tissue concentrations of oral maraviroc and raltegravir were lower in cervical and vaginal tissue than in rectal tissue.

In the same session (Session O-6), Fox and colleagues presented results of a phase IV study evaluating the pharmacokinetics and ex vivo potency of oral maraviroc against HIV challenge in 56 healthy men and women (Abstract 86LB). This study evaluated pharmacokinetics and pharmacodynamics after a single oral dose of maraviroc 300 mg, with the goal of informing event-driven dosing. Peak maraviroc levels were achieved after 4 hours in vaginal and rectal tissue, with vaginal and rectal tissue levels 3.6 times and 9.7 times, respectively, higher than the levels of maraviroc in plasma. In a tissue explant HIV-1 challenge model, there was a transient reduction in p24 antigen levels at 2 hours in vaginal tissue in women, however, this effect was lost by 4 hours after dosing. In rectal tissue, there were high levels of p24 antigen at all time points, suggesting no evidence of protection.

Although no protective effect was seen after a single dose, Fox noted that these findings do not preclude possible efficacy with repeated dosing of maraviroc, which is currently being evaluated in the HPTN 069 study. Similarly, in a poster presentation, Coll and colleagues evaluated the efficacy of single-dose oral maraviroc in preventing ex vivo HIV infection in a tissue explant model (Abstract 964). Rectal tissue was collected from 10 healthy MSM 4 hours after maraviroc dosing. Despite high maraviroc concentrations in rectal tissue, ex vivo HIV infection occurred in all participant specimens. In contrast, complete inhibition of infection was observed in cultures from 2 participants who received TDF and emtricitabine. In the audience discussion, it was mentioned that maraviroc can dissociate quickly in tissue, which may explain these findings. Boffito stressed the importance of optimizing and standardizing methods for evaluating the pharmacokinetics and pharmacodynamics of samples across studies.

Castillo-Mancilla and colleagues presented data on the use of emtricitabine triphosphate concentrations in DBSs as a marker of recent PrEP dosing (Abstract 83). Although tenofovir diphosphate in red blood cells is a marker of cumulative adherence (half-life, 17 days) and is highly predictive of efficacy, emtricitabine triphosphate reaches maximal concentrations at 4 hours (half-life, 31 hours), and could provide information on more recent dosing patterns. In an analysis of 515 paired plasma and DBS samples in iPrEx OLE, 94% of DBS samples were concordant for detection or nondetection of tenofovir and emtricitabine in plasma, another measure of recent drug exposure, demonstrating that emtricitabine triphosphate concentrations inform recent dosing.

Koenig and colleagues developed and validated a semiquantitative urine assay to measure adherence to PrEP with TDF (Abstract 975). In a series of cohort studies, investigators demonstrated a 100% concordance between presence of TDF in plasma and urine and detectability of tenofovir for more than 7 days in urine after a single dose of TDF and emtricitabine, compared with 2 days to 4 days in plasma. TDF in urine was cleared in a log-linear fashion, with a direct correlation to time since last dose. TDF concentrations of greater than 1000 ng/mL in urine were highly predictive of presence

of tenofovir in plasma, suggesting that the urine assay could distinguish between recent dosing within 48 hours (>1000 ng/mL), low adherence (>100 ng/mL), and no dosing within the past week (<10 ng/mL). Future work will be needed to develop the urine assay into a point-of-care test.

Finally, Gandhi and colleagues explored the use of hair and DBSs as markers of long-term PrEP adherence in iPrEx OLE (Abstract 514). In an analysis of 806 paired hair and DBS samples, there were strong and statistically significant correlations between tenofovir and emtricitabine levels in hair and tenofovir diphosphate and emtricitabine triphosphate levels in DBSs, and high levels of concordance (>80%) in drug detectability in the 2 matrices. As PrEP drug levels in DBSs were predictive of PrEP efficacy in iPrEx OLE, these findings suggest that hair concentrations could also be predictive of protection and that they warrant further evaluation as a tool to monitor adherence in PrEP programs.

Several presentations focused on comparison of different adherence measures used in PrEP clinical trials and evaluated correlates of adherence. Muganzi and colleagues compared several self-reported adherence measures, electronic monitoring, unannounced pill counts, and plasma tenofovir levels among 1143 participants in an ancillary adherence study within the Partners PrEP study (Abstract 976). All measures indicated high adherence: rating question (90%), frequency question (93%), percent question (97%), medication event monitoring system (MEMS; 97%), and unannounced pill counts (98%). For self-reported questions, the rating question "How well have you taken your study tablets?" had the widest distribution, suggesting this item may perform better at distinguishing between adherent and nonadherent individuals. However, no self-reported measure correlated closely with MEMS or unannounced pill counts, or discriminated between detectable and undetectable tenofovir drug levels, although MEMS performed best in distinguishing between detectable and undetectable drug levels. Amico and colleagues compared the performance of self-report with tenofovir drug detection in blood plasma in iPrEx OLE (Abstract 977). Among 1172 participants analyzed, the majority (84%) reported taking at least 1 dose in the past 3 days, 83% of whom had detectable drug levels. Among the 16% who reported not taking any doses in the past 3 days, 82% had undetectable drug levels. Sensitivity of self-report for drug detection was calculated as 96%, however, specificity was only 48%. Participants who were incorrectly assessed as adherent to PrEP by self-report were more likely to be younger, suggesting the need to develop alternative adherence measurement strategies in this population.

van der Straten and colleagues assessed rates and correlates of early tenofovir drug detection in plasma among oral tablet and gel users in the VOICE study (Abstract 979). Among 1146 participants who had plasma drug level testing at month 3, 34% had detectable tenofovir levels in the oral group and 27% had detectable drug in the gel group. Factors associated with drug detection in the oral group included being from Uganda or Zimbabwe, not receiving material support from a partner, no independent income, and alcohol use more than once per week. Factors associated with drug detection in the gel group included being from Zimbabwe, older age, not having a disapproving partner, not reporting any harm, no condom use at last sex, and no alcohol use (all $P < .05$). Having some risk perception was associated with drug detection in both groups. These results suggest that different factors may affect use of oral and topical agents, and highlight the importance of having different biomedical prevention options to address the varying prevention needs of women.

Roberts and colleagues evaluated whether intimate partner violence (IPV) is associated with low PrEP adherence among African women in the Partners PrEP study (Abstract 980). Among 1785 HIV-uninfected women, 16% reported IPV at 437 study visits: verbal, physical, and economic abuse were reported at 85%, 52%, and 37% of visits, respectively. Mean PrEP adherence rate as assessed by clinic pill counts was high (95%). In a multi-variable model, recent IPV (reported to have occurred in the past 3 months) was associated with a 42% higher likelihood of having low adherence (<80%) (aOR, 1.42; 95% CI, 1.09-1.86; $P = .01$). However, previous IPV was not associated with adherence ($P = .77$). The investigators recommend that if PrEP is to be prioritized for women who experience IPV, that the possibility of lower adherence be recognized, and that strategies to promote PrEP adherence be considered.

Potential Harms With PrEP

Several researchers evaluated potential harms associated with PrEP use, including renal toxicity, emergence of resistance, and medication sharing. Mugwanya and colleagues reported on the reversibility of kidney function decline among 3924 HIV-uninfected adults discontinuing PrEP in the Partners PrEP study (Abstract 981). Median time on study drug was 33 months. Mean estimated glomerular filtration rate (eGFR) was 2 mL/min/1.73 m^2 to 3 mL/min/1.73 m^2 lower at the last on-treatment visit among those taking PrEP than among those taking placebo ($P \leq .01$), and this difference was reversed by 4 weeks after discontinuation of study drug. The investigators conclude that declines in eGFR resolve within weeks after discontinuation of PrEP.

Kidney toxicity associated with PrEP with fixed-dose tenofovir disoproxil fumarate and emtricitabine is uncommon.

Panousis and colleagues reported on minor drug-resistant variants in the VOICE study (Abstract 982). Among 312 seroconverters in VOICE, it was previously reported that only 1 seroconverter had acquired the emtricitabine resistance–associated variant

M184V, and that none had acquired tenofovir resistance, as detected by standard population sequencing. Using an allele-specific polymerase chain reaction assay, 3 of 276 (1.1%) participants had the K65R mutation, none (0%) had the K70E mutation, 11 of 285 (3.9%) had the M184I mutation, and 2 of 288 (0.7%) had the M184V mutation. Overall, 15 of 289 (5%) seroconverters had low-frequency tenofovir and emtricitabine resistance, which was not associated with treatment arm or detection of tenofovir in blood. The investigators suggest that the low rate of product use in this trial likely explained the infrequent development of resistance mutations among seroconverters. Weis and colleagues evaluated the persistence of PrEP-selected drug resistance after discontinuation of drug in the Partners PrEP study (Abstract 983). It was previously reported that 9 of 121 seroconverters had PrEP-related drug-resistance mutations at detection of seroconversion. Using 454 ultradeep sequencing, all PrEP-selected mutations were no longer present at 6 months after HIV infection and study drug discontinuation, and remained undetectable at 12 months and 24 months. Penrose and colleagues evaluated cross-resistance between efavirenz or nevirapine and dapivirine, an investigational nonnucleoside analogue reverse transcriptase inhibitor currently being evaluated in intravaginal rings in 2 efficacy trials (Abstract 985). Among 102 plasma samples from individuals whose efavirenz- or nevirapine-containing treatment had failed, 77% showed an at least 10-fold resistance to dapivirine in in vitro testing. However, vaginal dapivirine concentrations seen with monthly ring use exceeded the adjusted 90% inhibitory concentration (IC_{90}) of the cross-resistant viruses by more than 23-fold. The researchers suggest that these high concentrations would likely prevent breakthrough infection of these viruses, although breakthrough could occur during a short window period following ring removal.

Naidoo and colleagues presented data on antiretroviral therapy outcomes among tenofovir gel users who acquired HIV infection and initiated therapy (Abstract 984). From 2011 to 2014, 59 participants who acquired HIV infection in a prior tenofovir gel–effectiveness trial were randomly assigned to receive a tenofovir-containing or -sparing regimen. Viral load suppression rates were not significantly different between the arms (86% and 78% in the tenofovir-containing and -sparing groups, respectively, at 12 months; $P = .68$), and median CD4+ cell count was similar between the groups. Women randomly assigned to receive the tenofovir-sparing regimen had a higher rate of grade 3 or 4 adverse events and had more toxicity-related regimen switches than those who received a tenofovir-containing regimen. Based on these findings, the researchers recommend tenofovir-containing regimens as the preferred treatment option for HIV-infected women with prior exposure to tenofovir gel.

Thomson and colleagues evaluated the extent of medication sharing among African, HIV-serodiscordant couples in the Partners PrEP study (Abstract 988). Drug sharing was reported at 4 of 137,462 (0.003%) HIV-uninfected partner study visits and at 1 of 12,601 (0.0008%) HIV-infected partner study visits, with a maximum of 2 to 4 tablets shared. Among 100 randomly selected HIV-infected partners not taking antiretroviral therapy, tenofovir was detected in plasma in only 1 individual who had an undetectable plasma viral load and tenofovir detected at enrollment and throughout follow-up, suggesting unreported use of tenofovir-containing antiretroviral therapy rather than sharing of PrEP. The researchers conclude that self-reported drug sharing appeared to be rare in this cohort and that concerns about drug sharing should not deter PrEP implementation, although ongoing monitoring outside of clinical trials is recommended.

PrEP Modeling

Several researchers modeled the potential population-level impact of PrEP rollout in different populations. Grant and colleagues presented data on current PrEP scale-up efforts in San Francisco, California, and their potential impact on HIV incidence (Abstract 25). Based on survey and surveillance data from 2014, it is estimated that approximately 16,089 HIV-uninfected men and women in San Francisco are behaviorally eligible for PrEP and that approximately one-third (5059 individuals, 31% of those eligible for PrEP) reported using PrEP in the past year. PrEP uptake appears to be strongly correlated with HIV risk behaviors, with 63% of respondents who had 6 or more sex partners reporting any PrEP use. Using a simple forecasting model assuming 62% viral suppression among HIV-seropositive persons in San Francisco, the investigators estimated that a 70% reduction in HIV infections could be achieved if PrEP uptake expands approximately 3-fold in San Francisco and increases proportionally according to risk (up to 95% uptake in the highest risk strata). According to this model, further increases in viral suppression rates would have additive population-level benefits.

Bernard and colleagues modeled the benefits of PrEP scale-up among IDUs in the United States (Abstract 1121). Using a dynamic compartment model of HIV prevalence in the United States, capturing both sexual and injection transmission as well as overlap between these groups, they estimated that providing PrEP to 50% of IDUs could reduce HIV prevalence from 6.2% to 4.2%. The cost-effectiveness of PrEP for IDUs depends greatly on PrEP adherence and efficacy, and whether it is delivered to the subgroups at highest risk. Frequent HIV screening and timely linkage to treatment also enhance the value of PrEP.

Ying and colleagues evaluated the cost-effectiveness of delivering PrEP to high-risk, HIV-serodiscordant couples as a bridge to antiretroviral therapy in the Partners Demonstration Project (Abstract 1106). The annual cost of PrEP delivery was $1,058 per couple in the study and an estimated $453 in public health settings (based on public

sector salaries, lower medication and testing costs, and task shifting). Compared with current spending on couple-based strategies, implementing PrEP as a bridge to viral suppression would cost less than $100 per couple, and the majority of costs are attributed to increasing antiretroviral therapy coverage among HIV-seropositive individuals. The investigators conclude that this PrEP strategy may be cost-effective and could avert 17% of new HIV infections, but broader coverage of PrEP and antiretroviral therapy would be required to further drive down infections.

Mabileau and colleagues modeled the cost-effectiveness of several different strategies to prevent HIV transmission in fertile, heterosexual, HIV-serodiscordant couples in which the woman is HIV-uninfected, the man is HIV-infected and taking antiretroviral therapy with a suppressed viral load, and who want to have a child (Abstract 1122). The model included HIV care costs for both the woman and her child, if born HIV-infected. The likelihood of HIV transmission was highest with unprotected sexual intercourse (treatment-as-prevention approach) and lowest for medically assisted procreation (intrauterine insemination with sperm washing). Targeting unprotected sex on fertile days was associated with the lowest cost. Use of PrEP during the fertile period could further lower HIV transmission risk but had an unfavorable cost-effectiveness ratio compared with unprotected sex alone on fertile days (incremental cost-effectiveness ratio 1,130,000/life-year saved). However, the investigators conclude that PrEP use during the fertile period could become very cost-effective if the cost of PrEP is lowered.

Two investigations evaluated the impact of STIs on HIV risk and PrEP efficacy in macaques. Radzio and colleagues reported results of a vaginal challenge study among 11 female macaques inoculated with Chlamydia trachomatis and Trichomonas vaginalis and exposed weekly to SHIV (Abstract 962). Although all 5 placebo-receiving control animals became infected, 4 of 6 animals treated with TDF and emtricitabine remained uninfected after 16 challenges. Tenofovir diphosphate and emtricitabine triphosphate concentrations in peripheral blood mononuclear cells were similar between the protected and infected macaques. However, animals infected with Chlamydia trachomatis or Trichomonas vaginalis may have higher deoxyadenosine triphosphate and deoxycytidine triphosphate levels in vaginal tissues, thought to be caused by increased cellular activation. The investigators conclude that TDF and emtricitabine maintains efficacy in a macaque model of prolonged coinfection with STIs, although biologic changes caused by persistent STI infections may lower the protection threshold.

Similarly, Makarova and colleagues evaluated the impact of STIs on the efficacy of 1% tenofovir vaginal gel in 10 female macaques (Abstract 963). All SHIV-challenged macaques receiving placebo became infected, all 6 animals treated with tenofovir gel 30 minutes before exposure remained uninfected after 20 challenges, and tenofovir gel applied 3 days before exposure protected 3 of 6 macaques. Evaluation of longitudinal plasma samples showed statistically significantly higher peak tenofovir plasma drug levels in STI-infected animals than in non–STI-infected animals, which may reflect increased tissue permeability and drug loading.

PEP

In a themed discussion, several researchers presented new data on the safety and tolerability of different PEP regimens (Session TD-V). Mayer opened the session by reviewing the revised World Health Organization (WHO) guidelines on PEP released in 2014.[20] Key points included 1) not differentiating between occupational and nonoccupational exposures; 2) that 3-drug PEP regimens are preferred over 2-drug regimens, although 2-drug regimens are also effective; 3) that an entire 28-day course of PEP should be prescribed following initial risk assessment, to facilitate course completion; and 4) that adherence counseling and support should be provided to all individuals starting PEP.

The World Health Organization released new guidelines on PEP in 2014.

The guidelines recommend TDF plus lamivudine (or emtricitabine) combined with boosted lopinavir or atazanavir as the preferred PEP regimen for adults and adolescents, although raltegravir, boosted darunavir, or efavirenz could be considered as the third drug. For pediatric populations, zidovudine plus lamivudine is recommended with ritonavir-boosted (/r) lopinavir for children aged 10 or younger, with alternative regimens listed for older children.

Leal and colleagues reported results from 2 randomized trials comparing lopinavir/r, the standard of care, with maraviroc or raltegravir, both taken with TDF and emtricitabine, among patients presenting to the ED after potential sexual exposure to HIV (Abstract 959). PEP discontinuation rates before day 28 were significantly higher in the arm receiving lopinavir/r (31.5%) than in the arm receiving maraviroc (11.6%) ($P = .001$), and in the arm receiving lopinavir/r (36.6%) than in the arm receiving raltegravir (23.7%). The proportion of participants with low PEP adherence was similar in the lopinavir/r and maraviroc groups (54% vs 46%, respectively; $P = .56$), but was higher in the lopinavir/r than in the raltegravir group (49.2% vs 30.8%, respectively; $P = .03$). Rates of adverse events were also higher in the lopinavir/r arms in both studies ($P < .05$). In a multivariable analysis, lopinavir/r-containing PEP regimens and non-Caucasian race were associated with higher PEP discontinuation rates.

Foster and colleagues presented results of a nonrandomized, open-label study of TDF and emtricitabine coformulated with rilpivirine as a single-tablet PEP regimen (Abstract 958). Among 100 men who initiated PEP in Australia, 92% completed the course,

with 98.5% adherence by self-report and 98.6% adherence by pill count in those who completed the 28-day course. Plasma tenofovir levels were greater than 40 ng/mL (consistent with full adherence) in 88% of participants tested at day 28. Overall, 4% of participants experienced a grade 3 or 4 laboratory or clinical adverse event attributable to the study drug. The investigators conclude that this single-tablet regimen was well tolerated, with patients achieving high levels of adherence and completion.

New PEP regimens may offer lower toxicity and result in higher PEP course completion rates.

In a retrospective study between 1996 and 2014, Wiboonchutikul and colleagues described the characteristics of occupational HIV exposures among health care workers and the factors associated with not completing a 4-week PEP course (Abstract 957). Among 225 exposures, 163 resulted from percutaneous injury, 43 from mucosal exposure, 6 from nonintact skin exposure, and 13 from intact skin exposure. Nurses were most frequently exposed (43%), followed by patient or nurse assistants (18%), and medical technicians (15%). PEP was initiated in 155 episodes and was subsequently intentionally discontinued in 26 episodes. Of the remaining 129 health care workers, only 71% completed the 4-week PEP course. In a multivariable analysis, use of a regimen with 2 nucleotide analogue reverse transcriptase inhibitors with efavirenz was the only factor associated with not completing the PEP course (OR, 37.8; 95% CI, 4.2-342.3), and drug was discontinued owing to intolerability in all cases. The researchers recommend that this regimen no longer be used as occupational PEP in resource-limited settings.

Efavirenz-based PEP regimens are associated with greater rates of medication discontinuation for occupational PEP and should be avoided in these settings.

Haidari and colleagues presented data from a case series of individuals diagnosed with acute HIV infection after initiation of PEP in the United Kingdom (Abstract 961). In a multicenter, retrospective, case-note review, 19 patients were identified as a PEP failure (1 case) or delayed diagnosis of acute HIV infection (18 cases). All patients accessed PEP within 72 hours and initiated triple therapy with 2 nucleotide reverse transcriptase inhibitors and a boosted protease inhibitor, per local guidelines. Of 18 patients diagnosed with HIV infection while taking PEP, 11 elected to continue antiretroviral therapy once HIV diagnosis was confirmed and 6 chose to discontinue PEP. Two of 17 individuals who had baseline drug resistance testing showed evidence of substantial drug resistance mutations (K103N, T215D) that was likely transmitted resistance unrelated to PEP. The investigators recommend that all point-of-care tests be used in parallel with a fourth-generation test and that HIV polymerase chain reaction (PCR) be considered, to reduce missed acute infections. They also suggest that dual therapy be avoided, to prevent the emergence of resistance. Further, in the setting of an acute HIV infection diagnosis after PEP initiation, they recommend continuing antiretroviral therapy until urgent review by an HIV specialist, as continuing early anti-retroviral therapy initiation may have potential benefits in limiting the viral reservoir.

HIV testing methods able to detect acute infection (fourth-generation tests, HIV RNA) are recommended in the setting of PEP initiation.

During the audience discussion, questions were raised about the need for a full 28-day course of PEP. Data from nonhuman primates suggest that a shorter duration may be possible if PEP is initiated very quickly after exposure. Given the lack of data, there was a call for studies to evaluate different durations of PEP regimens. Another question raised was whether PEP delays antibody development in individuals who seroconvert while taking PEP, for which there are few data. It was discussed that having a national registry of PEP cases could help address some of these issues. Finally, it was mentioned that PEP initiation provides an important opportunity to discuss the appropriateness of PrEP, particularly for patients who have recurrent HIV exposures or take repeated PEP courses.

Medical Circumcision

In a symposium session on scale-up of interventions (Session S-8), Thomas, who presented on behalf of Reed, reviewed data supporting medical circumcision (MC), including 3 randomized trials showing an approximately 60% efficacy rate of reducing HIV acquisition (Abstract 175). Based on these results, WHO issued guidance on MC for HIV prevention in 2007. Modeling studies conducted in 2011 indicate that reaching 80% circumcision coverage (approximately 20 million circumcisions) could avert 3.4 million HIV infections and have a net cost savings of US $16.5 billion within 15 years, with 1 HIV infection averted for every 9 men circumcised. To achieve successful MC scale-up, key areas of focus have included: fostering community engagement, establishing complex supply chains, conducting clinician trainings, and launching demand-creation campaigns. Thomas presented several lessons learned from MC trials and implementation.

First, loss-to-follow-up rates appear to be higher in MC programs than in randomized controlled trials, and adverse event rates among those lost to follow-up also appear to be higher. In response, there have been increased efforts to minimize loss to follow-up in MC programs, and US President's Emergency Plan for AIDS Relief (PEPFAR) has instituted mandatory reporting of all deaths and specific MC-related adverse events. MC programs also provide a unique opportunity to engage men who are not already engaged in the health system, by offering HIV testing services and linkage to care for individuals who

test HIV-seropositive. Reaching those in the highest incidence groups will help avert the greatest number of HIV infections. Further, modeling studies have indicated that circumcising younger men, aged 15 years to 24 years, can achieve substantial population-level impact with fewer MC procedures.

MC programs can foster engagement of men in the health care system and encourage HIV testing and linkage to care for HIV-infected men.

In an oral abstract session on demonstrating impact (Session O-13), Kong and colleagues presented data on the impact of MC scale-up on community-level HIV incidence in Rakai, Uganda (Abstract 158). Median MC coverage was 4% prior to randomized trials (from 1999-2003), 9% during the trials (from 2004-2007), and 26% in the posttrial period (from 2008-2011). For every 10% increase in MC coverage, there was a 12% reduction in community-level HIV incidence among non-Muslim men, a robust finding after adjusting for concurrent increases in antiretroviral therapy coverage in women, age, and sexual risk behaviors. However, no association was found between MC coverage and community-level HIV incidence among non-Muslim women. Kong hypothesized that this lack of association among women, which is in contrast to modeling predictions, may have been attributable to the relatively short follow-up period.

Medical MC was scaled up to more than 6 million individuals in 2013.

In a themed discussion on voluntary MC for HIV prevention (Session TD-Y), Njeuhmeli presented data on the rollout of MC, with more than 6 million circumcisions performed between 2008 and 2013 and rates doubling over the last 2 years. He highlighted key considerations for scaling up of MC, including the age profile of MC clients, human resource constraints, potential for risk compensation, and resumption of sex before wound healing.

Hellar and colleagues described outcomes from MC scale-up strategies in Tanzania (Abstract 1086). MC is being provided through routine, larger health facilities and through campaigns where teams of practitioners move into new communities and do a high volume of MCs in 1- to 3-week bursts. In 2014, mobile services were launched to serve hard-to-reach areas using a roving team of practitioners to provide MC services, often in nonfacility settings. Among 148,880 persons circumcised between October 2013 and August 2014, 76% were younger than 20 years of age. Mobile teams reached more than 5000 individuals who were more likely to be older (≥20 years of age) than those reached by other service-delivery models, and it was noted that mobile outreach provides more privacy for older clients. Follow-up rates were high in the mobile outreach setting, likely because of active client follow-up. The investigators conclude that mobile services could be an efficient strategy to engage with older MC clients.

Rugwizangoga and colleagues presented data on the acceptability of a device for nonsurgical MC (PrePex™) when used in routine, programmatic settings in Rwanda (Abstract 1087). This device offers an alternative to conventional surgery and does not require injectable anesthesia or cutting of tissue. Between 2009 and 2014, 86,284 adolescent boys and adult men were circumcised at program sites, and nearly two-thirds (63%) of circumcisions have used the nonsurgical device since its introduction in February 2014. Overall uptake of MC has increased yearly, with a doubling of the number of clients served from 2012 to 2013. This trend has been attributed to increased efficiencies, including shifting of the task to nurses, use of mobile outreach teams, and the introduction of the nonsurgical device, although the program experienced a stock-out of certain sizes of the device in July 2014, which limited scale-up efforts. The investigators conclude that the device is well accepted by MC clients in Rwanda and recommend that programs ensure adequate availability of the device in all sizes.

Kagaayi and colleagues described characteristics of MC acceptors, risk compensation, and effectiveness in Rakai, Uganda (Abstract 1088). The researchers compared 1192 MC acceptors with 2384 nonacceptors who participated in the Rakai community cohort surveys between 2007 and 2013. MC acceptors were younger, less likely to be married, had higher education levels, and were more likely to have reported genital ulcers ($P = .006$). After MC, sexual activity increased by 3% per year among MC acceptors, an increase that was more prominent among younger men. Sexual activity with women in occupations with higher risk (eg, bar attendants, alcohol brewers, restaurant workers, traders, fisherfolk, housemaids) increased by 10.2% per year among MC acceptors, with no change among uncircumcised men. However, HIV incidence was lower among MC acceptors (0.61/100 person-years) than among nonacceptors (1.11/100 person-years) (incidence rate ratio 0.50; $P = .05$). These data suggest that men at higher risk self-selected to receive MC, possibly because they were told MC reduces risk for genital ulcer disease. Although there was some evidence of risk compensation in this cohort, this did not attenuate the effectiveness of MC. During the audience discussion, the speakers discussed strategies to increase demand for MC, including use of community mobilizers and peer promoters, radio and media campaigns, and new communication strategies to promote the attractiveness of MC in addition to its potential health benefits.

Although MC decreases risk for genital ulcers and human papilloma virus acquisition in HIV-infected men, a previous study showed that MC increased the rate of HIV transmission to female partners if sex was resumed prior to wound healing. In an oral abstract session on prevention (Session O-1), Manucci and colleagues presented data on penile HIV shedding after MC in HIV-infected men (Abstract 30). In a prospective

study of 236 HIV-seropositive men undergoing MC in Rakai, Uganda, increased HIV shedding was observed 1 week to 2 weeks after MC, but was lower after wound healing at weeks 6 and 12. Among antiretroviral treatment–naive men, HIV shedding decreased from 8.8% at enrollment to 1.9% at month 3 (prevalence rate ratio, 0.19; 95% CI, 0.06-0.64). Further, suppressive antiretroviral therapy decreased the number of HIV shedding events and penile HIV viral load. These results suggest the potential long-term benefit of reduced penile HIV shedding after MC in HIV-seropositive men. The investigators highlight the importance of preventing HIV transmission during the wound-healing period and propose that initiation of antiretroviral therapy at the time of MC should be considered in order to reduce HIV transmission risk.

HIV and HSV-2 shedding are increased in the weeks following MC, and sexual abstinence is crucial during this period of wound healing.

Grabowski and colleagues presented data on HSV-2 shedding among 176 men coinfected with HIV and HSV-2 undergoing MC (Abstract 1084). HSV-2 shedding was detected in 9.7% of men prior to surgery, with a nonstatistically significant increase to 14.8% at week 2 ($P = .153$) that then decreased to 6.9% by week 6 ($P = .33$). HSV-2 shedding was noted to be 39% lower among men with healed MC wounds ($P = .08$). HSV-2 viral load was also somewhat higher at week 1 post-MC. The investigators recommend that MC programs should provide counseling on sexual abstinence during wound healing and use of condoms thereafter.

Strategies to Reduce HIV Transmission From HIV-Seropositive Individuals

HIV-Serodiscordant Couples

Mujugira and colleagues presented data on the impact of antiretroviral treatment on HIV transmission from an HIV-infected person to a partner of the opposite sex in the placebo arm of the Partners PrEP study (Abstract 989). HIV incidence among 496 HIV-uninfected partners was roughly equivalent whether the HIV-seropositive partner was eligible for but had not initiated antiretroviral therapy (1.71/100 person-years; 95% CI, 0.35-5.01), or if the HIV-seropositive partner had initiated antiretroviral therapy within the previous 6 months (1.79/100 person-years; 95% CI, 0.37-5.22). There were no transmissions during 167 person-years of follow-up, after 6 or more months of antiretroviral therapy (0.0/100 person-years; 95% CI, 0.00-2.20). This suggests that there is residual risk of HIV transmission during the first 6 months of treatment after antiretroviral therapy, and that other prevention services should be provided during this time, including PrEP.

HIV transmission may continue to occur up to 6 months after antiretroviral treatment, after which transmission may be rare.

Grulich and colleagues report interim results on 234 HIV-serodiscordant couples of MSM enrolled from Australia, Thailand, and Brazil (Abstract 1019LB). At baseline, 84% of the HIV-seropositive partners were taking antiretroviral therapy and 83% had undetectable plasma HIV RNA levels (defined as < 200 copies/mL). To date, there have been no linked HIV infections from 5905 condomless sex acts. The upper 95% confidence limit for risk of HIV transmission among these couples was 4.06 per 100 couple-years of follow-up for condomless anal sex and 6.46 per 100 couple-years for condomless receptive anal sex. Although this provides further encouraging news about reduced risk of HIV transmission from virally suppressed HIV-infected individuals, additional follow-up of these couples will provide greater certainty, with smaller CIs, about the risk of transmission.

Chohan and colleagues presented phylogenetic data from 458 HIV-serodiscordant couples in Nairobi, Kenya (Abstract 1035). As has been demonstrated in other studies, a substantial minority of HIV infections of the HIV-seronegative partner originate outside the couple. The investigators reported that only 8 of 12 HIV infections were phylogenetically linked. These data reinforce the need for prevention strategies for HIV-seronegative individuals that extend to relationships outside of known HIV-serodiscordant partnerships.

Serosorting

A themed discussion was held on the topic of serosorting, with general consensus that better measures are needed to differentiate between reported risk behaviors and intentional serosorting—choosing sex partners or practices based on the perceived seroconcordance or serodiscordance of one's partner (Session TD-W). All of the data presented in this themed discussion dealt with reported seroconcordant or serodiscordant behavior among MSM, without knowledge of whether individuals were choosing partners and practices based on serostatus. Paz-Bailey and colleagues presented data on temporal trends from the NHBS system in the proportion of MSM reporting HIV-seroconcordant or -serodiscordant condomless anal sex from 2005 to 2014 (Abstract 1060). They reported that among HIV-seronegative MSM, -seroconcordant and -serodiscordant condomless anal sex increased among all races and ethnicities ($P < .01$) and age groups ($P < .001$). Among HIV-seropositive men, -seroconcordant condomless anal sex increased ($P = .001$). Khosropour and colleagues presented data from 2 retrospective cohorts of MSM attending an STI clinic in Seattle from 2002 to 2012 (Abstract 1061). They compared 186 HIV-seroconverters and 1000 HIV-seronegative controls, and compared prediagnosis with postdiagnosis visits. The proportion of serosorters remained stable in both cohorts; however, after diagnosis, men who seroconverted switched from engaging in condomless anal sex with

HIV-seronegative partners to engaging in the same sexual practice with HIV-seropositive partners. This emphasizes the importance of early diagnosis of newly HIV-infected persons to reduce HIV transmission before diagnosis.

Treatment as Prevention

Gardner and colleagues presented data on more than 14,000 HIV-infected patients of clinics in 6 US cities (Abstract 101). They evaluated the proportion of time (April 2009-March 2013) during which these patients had HIV RNA levels of greater than 1500 copies/mL, a threshold associated with HIV transmission in epidemiologic studies. Despite the fact that more than 90% of patients were taking antiretroviral therapy at the time the study was initiated, an HIV RNA level of greater than 1500 copies/mL was observed during 23% of the follow-up time period. Younger patients; black patients; those with longer intervals between viral load testing; those whose care was funded by Medicaid, by the Ryan White HIV/AIDS Program, or by a charity care program; and persons not taking antiretroviral therapy were more likely to have HIV RNA levels above the 1500 copies/mL threshold during the study. MSM were less likely to have viral loads above the threshold. The investigators suggest that particular attention be paid to those patients with more than 6 months between viral load tests, to ensure full viral suppression and reduce the risk of HIV transmission.

Tanser and colleagues presented on the use of population viral load to predict HIV incidence using data from the Africa Centre Demographic Information System in rural KwaZulu-Natal, South Africa (Abstract 991LB). Investigators observed 642 seroconversions across nearly 25,000 person-years of observation. There was no association between the geometric mean viral load within a population and the risk of HIV acquisition among HIV-negative individuals living in that community ($P = .49$). However, every 1% increase in the proportion of the entire population (irrespective of HIV serostatus) with detectable virus was associated with a 4.9% increase in individual risk of HIV acquisition ($P < .001$). This suggests that population-wide measures that take into account the spatial variations in population prevalence may provide helpful measures for targeting interventions within communities.

Moore and colleagues presented data on 719 MSM recruited in Vancouver, Canada, using respondent-driven sampling (Abstract 1023). HIV prevalence was 23.4% overall, 98% of individuals were aware of their HIV infection, 93% were taking antiretroviral therapy, and 81.4% were virally suppressed. However, in multivariate analysis, lack of viral suppression was associated with a greater likelihood of engaging in condomless sex with a partner who was HIV seronegative or whose serostatus was unknown (aOR, 3.13; 95% CI, 1.1-8.9). This serves as a reminder that both behavioral and biomedical intervention are needed to reduce HIV transmission from HIV-infected persons.

Other Prevention Strategies

Cash Transfers

Wilson presented data on the impact of social protection programs that address poverty on health, income, and HIV risk (Abstract 75). He pointed out that almost 25% of the world live on less than $1.25 per day, and 50% live on less than $2.50 per day. He presented evidence from 3 randomized controlled trials of how cash transfers affected HIV and STI acquisition in 3 African countries. In a study of nearly 2400 individuals aged 18 years to 30 years in Tanzania in which participants were randomly assigned to receive 1 of 2 levels of conditional cash transfers if they remained STI free, there was a 25% decrease in STIs (combined HIV, HSV-2, and syphilis) among those receiving the higher level cash transfer of $60 annually. In a study of nearly 1300 young women in Malawi (aged 13 years-22 years), participants were given either conditional (mandatory school attendance) or nonconditional cash transfers (≤$15/month). Overall, there was a 60% decrease in HIV prevalence and a 76% reduction in HSV-2 infections. Women in the study were also 33% less likely to be sexually active, had a 25% reduction in number of sex partners, and had a 30% lower rate of teen pregnancy. There was no substantial difference between those who received a conditional cash transfer and those who received a nonconditional cash transfer. A study of more than 3200 young men and women aged 18 years to 32 years in Lesotho randomly assigned participants to receive 1 of 2 tiers of lottery prizes ($50 or $100, awarded quarterly) and resulted in a 25% reduction in HIV incidence overall, with somewhat better results among younger girls and those in the $100 arm. Wilson concluded by mentioning 2 additional cash transfer studies that will have data available in the next year, which will add considerably to understanding of the generalizability of this approach in decreasing new HIV infections.

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.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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Review

CROI 2015: Advances in Antiretroviral Therapy

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The 2015 Conference on Retroviruses and Opportunistic Infections included new and exciting advances in the realm of antiretroviral therapy. The Temprano trial demonstrated benefits from early antiretroviral therapy and isoniazid preventive therapy. Important data on investigational antiretroviral drugs were presented, including tenofovir alafenamide fumarate and BMS-955176, an HIV-1 maturation inhibitor. Novel data on the HIV care continuum from resource-rich and -limited settings highlighted persistent sex- and race-related disparities in care engagement, and the crucial need to bring HIV testing and care into the community to improve engagement across the care continuum. Life expectancy data from resource-limited settings reveal dramatic improvements across sub-Saharan Africa, although people with HIV still live 5 years to 10 years less than those without HIV, and new cost-effectiveness research revealed that the price of antiretroviral therapy itself remains a key driver of cost and cost-effectiveness calculations. Results from the PROMISE trial showed reduced rates of mother-to-child transmission among women who received antiretroviral therapy with 3 drugs compared with women who received zidovudine monotherapy, supporting current World Health Organization guidelines.

Keywords: CROI 2015, HIV, antiretroviral therapy, cure, care cascade, resource-limited settings, mother-to-child transmission, MTCT, resistance

New Antiretroviral Agents

Maturation Inhibitors

At the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 23 to 26, Lataillade and colleagues presented data on BMS-955176, an investigational HIV-1 maturation inhibitor (Abstract 114LB). BMS-955176 blocks the cleavage between capsid protein p24 and spacer protein 1 in Gag. This compound is active against a broad range of isolates that are resistant to the first-generation investigational maturation inhibitor bevirimat, which did not complete clinical development. The investigators conducted a proof-of-concept, dose-ranging study of HIV-infected adults not taking antiretroviral therapy. Participants received 10 days of monotherapy with BMS-955176 at the assigned dose or a placebo. They found similar antiviral activity with the 3 highest doses (40 mg, 80 mg, and 120 mg once daily) of approximately 1.6 $\log_{10}$ copies/mL reductions in plasma HIV RNA levels. Baseline polymorphisms in Gag did not appear to impair antiviral efficacy. There were no safety concerns identified. These data support further clinical development of this compound. Jeffrey and colleagues presented data on another investigational HIV-1 maturation inhibitor, GSK 2838232 (Abstract 538). The investigators found that this compound was active against a broad range of isolates, including those resistant to bevirimat.

Broadly Neutralizing HIV-1 Antibodies

Bolton and colleagues presented data on the use of broadly neutralizing HIV-1 antibodies to treat acute simian-human immunodeficiency virus (SHIV) infection in rhesus macaques (Abstract 50). The investigators compared changes in viremia during standard antiretroviral therapy, treatment with a single monoclonal antibody, treatment with a combination of antibodies, and no treatment. A combination of antibodies produced similar declines in viremia to those seen with standard antiretroviral therapy. These data support the continued development of broadly neutralizing HIV-1 antibodies for potential clinical use.

ABX464

Tazi and colleagues presented data on ABX464, an investigational compound that targets Rev functions (Abstract 104LB). Rev inhibits splicing of viral messenger RNA (mRNA) and helps to export mRNA from the nucleus to the cytoplasm. ABX464 enhances viral mRNA splicing by interfering with these Rev-mediated functions. ABX464 does not interfere with normal cellular processing of mRNA. ABX464 demonstrated antiviral activity in a humanized mouse model. Early human trials suggest that the compound is safe. A proof-of-concept trial in HIV-infected adults is underway.

Clinical Trials of Antiretroviral Therapy

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide Fumarate

Wohl and colleagues presented data on a single-tablet regimen containing the investigational drug tenofovir alafenamide fumarate (TAF) (Abstract 113LB). TAF is a new prodrug of tenofovir that

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results in higher intracellular levels of tenofovir diphosphate but lower plasma levels of tenofovir than does tenofovir disoproxil fumarate (TDF), thus leading to reduced bone and renal toxicity. The investigators combined data from 2 identical-phase clinical trials that enrolled HIV-infected antiretroviral treatment–naive adults with plasma HIV-1 RNA levels of at least 1000 copies/mL and estimated creatinine clearance levels of 50 mL/min or higher. Participants were randomly assigned to receive a single-tablet regimen of elvitegravir, cobicistat, emtricitabine, and TDF (n = 866) or of elvitegravir, cobicistat, emtricitabine, and TAF (n = 867).

Baseline characteristics of the study population included female sex (15%), black race (26%), Hispanic race (19%), a median HIV-1 RNA level of 4.58 $\log_{10}$ copies/mL, and a median CD4+ count of 405 cells/μL. The primary endpoint was a plasma HIV-1 RNA level of less than 50 copies/mL at week 48. This was achieved by 92% of individuals in the TAF-containing arms and 90% of individuals in the TDF-containing arms (difference, 2.0%; 95% confidence interval [CI], -0.7-4.7%), thus meeting protocol-defined noninferiority. This treatment effect was consistent across various subgroups. The emergence of resistance was low, occurring in 7 (0.8%) individuals in the TAF-containing arms and 5 (0.6%) individuals in the TDF-containing arms. Among these participants, all developed the M184V/I resistance mutation, 3 developed the K65R mutation, and 8 developed integrase resistance mutations. Adverse events leading to treatment discontinuation occurred in 8 (0.9%) individuals in the TAF-containing arms and 13 (1.5%) individuals in the TDF-containing arms. Both regimens appear to be safe and well tolerated.

Sax and colleagues presented data on renal and bone safety from these same clinical trials (Abstract 143LB). In a pharmacokinetic subset from these trials, plasma concentrations of tenofovir were 90% lower in the TAF-containing arms, and intracellular tenofovir diphosphate concentrations were 4.1 times higher, confirming data from earlier studies. Both arms had early declines in estimated glomerular filtration rate consistent with the known effect of cobicistat on renal tubular secretion of creatinine. Through week 48, this decrease was greater in the TDF-containing arms than in the TAF-containing arms (-11.2 mL/min vs 6.6 mL/min; $P < .0001$). There were 4 renal events leading to treatment discontinuation in the TDF-containing arms and none in the TAF-containing arms. The investigators examined changes in quantitative proteinuria between arms using several different markers. All of these analyses showed significantly greater proteinuria in the TDF-containing arms than in the TAF-containing arms ($P < .001$).

Sax and colleagues also reported on changes in bone mineral density using dual-energy X-ray absorptiometry (DXA). They found that the loss of bone mineral density was significantly greater in the TDF-containing arms than in the TAF-containing arms in the spine (-2.86% vs -1.30%; $P < .001$) and the hip (-2.95% vs. -0.66%; $P < .001$). Lipid levels (total cholesterol, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and triglyceride levels) were higher at week 48 in the TAF-containing arms than in the TDF-containing arms, but ratios of total cholesterol levels to HDL cholesterol levels were similar. These studies confirm prior findings about potential advantages of TAF over TDF for bone and renal toxicity. The single-tablet regimen of elvitegravir, cobicistat, emtricitabine, and TAF was submitted for US Food and Drug Administration (FDA) approval based on these data.

Cabotegravir and Rilpivirine

Margolis and colleagues presented follow-up data from a study examining an oral combination of the investigational integrase strand transfer inhibitor (InSTI) cabotegravir with rilpivirine as maintenance antiretroviral therapy compared with efavirenz and 2 nucleoside analogue reverse transcriptase inhibitors (nRTIs) (Abstract 554LB). This 2-drug, oral maintenance therapy generally maintained virologic suppression from week 48 to week 96. These data support the continued development of these 2 drugs as oral or injectable maintenance therapy.

Fozivudine

Kroidl and colleagues conducted a clinical trial of fozivudine in HIV-infected adults in Tanzania and Cote d'Ivoire (Abstract 544). Fozivudine is an investigational thioether lipid–zidovudine conjugate that was designed to have less toxicity and better pharmacokinetics than zidovudine. Fozivudine is converted to the same active form as zidovudine. Investigators randomly assigned participants to receive 1 of 3 doses of fozivudine or a zidovudine control, each given with lamivudine and efavirenz. The antiviral activity appeared comparable between groups. There were fewer early declines in hemoglobin value and neutrophil count in the fozivudine group, but there were no differences by week 24. There was no difference in treatment discontinuation rates owing to adverse effects. It is not clear whether these results support further development of this compound.

Antiretroviral Therapy Strategies

Early Initiation of Antiretroviral Therapy and Isoniazid Preventive Therapy

Danel and colleagues presented data from Temprano, a large randomized clinical trial investigating the optimal time to start antiretroviral therapy and whether isoniazid preventive therapy (IPT) was effective for HIV-infected adults from Cote d'Ivoire (Abstract 115LB). Eligible participants had a CD4+ count of less than 800 cells/μL and were not otherwise eligible for antiretroviral therapy. World Health Organization (WHO) CD4+ count criteria for antiretroviral therapy initiation shifted from 200 cells/μL to 350 cells/μL to 500 cells/μL during the course of the study.[1] Participants were randomly assigned to 1 of 4 arms:

immediate antiretroviral therapy with IPT, immediate antiretroviral therapy without IPT, antiretroviral therapy based on WHO criteria with IPT, and antiretroviral therapy based on WHO criteria without IPT. In the IPT arms, participants received 6 months of daily isoniazid. The initial antiretroviral regimen was efavirenz, TDF, and emtricitabine with the option of replacing efavirenz with ritonavir-boosted (/r) lopinavir or zidovudine if clinically indicated. The primary outcome was time to severe HIV morbidity (death, AIDS-defining illness, serious bacterial illness or non–AIDS-related cancer). Two thousand fifty-six participants were randomly assigned and included in the analysis. Baseline characteristics of study participants included female sex in 78%, a median age of 35 years, WHO stage 1 or 2 HIV disease in 90%, a median CD4+ count of 465 cells/μL, a median HIV-1 RNA level of 4.7 $\log_{10}$ copies/mL, and a positive tuberculosis test result in 35%. The median length of follow-up was 29.9 months. Among those randomly assigned to antiretroviral therapy based on WHO criteria, 58% started antiretroviral therapy in follow-up after a median of 14.8 months. Eighty-five percent of those randomly assigned to IPT completed 6 months of isoniazid.

There was no interaction between IPT and time of antiretroviral therapy initiation, allowing the investigators to analyze these separately. Early antiretroviral therapy reduced the risk of the primary endpoint by 44% ($P = .00020$), and IPT reduced the risk by 35% ($P = .005$). The investigators presented a secondary analysis restricted to those who entered the study with a CD4+ count of 500 cells/μL or higher. The efficacy estimates were similar to those in the primary analysis. Participants randomly assigned to early antiretroviral therapy experienced a higher rate of grade 3 or 4 adverse events in the first 6 months postrandomization, with no observed difference thereafter. IPT was not associated with increased grade 3 or 4 adverse events. Overall, this study provides strong support for the current WHO recommendations for IPT and initiation of antiretroviral therapy at CD4+ counts of 500 cells/μL or less.[2]

Monotherapy With a Protease Inhibitor

Hakim and colleagues presented 144-week data from the EARNEST (Europe-Africa Research Network for Evaluation of Second-line Therapy) trial, which randomly assigned HIV-infected African adults whose initial nonnucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen had failed, to receive lopinavir/r plus 2 nRTIs, lopinavir/r plus raltegravir, or 12 weeks of lopinavir/r plus raltegravir followed by lopinavir/r alone (Abstract 552). After a median follow-up of 124 weeks, the data and safety monitoring board recommended that an nRTI be reinitiated in the arm receiving lopinavir/r alone. Higher rates of viremia were observed in this arm that resolved after nRTI reinitiation. More participants in the arm receiving lopinavir/r alone developed intermediate- to high-level lopinavir resistance than did those in the arms receiving lopinavir/r plus 2 nRTIs or lopinavir/r plus raltegravir (11.0% vs 2.4% and 2.7%, respectively). The investigators concluded that lopinavir/r plus 2 nRTIs should remain the treatment of choice for second-line antiretroviral therapy in resource-constrained settings.

Ripamonti and colleagues combined data from 2 clinical trials to examine predictors of sustained viral suppression during monotherapy with darunavir/r as maintenance antiretroviral therapy (Abstract 551). The investigators found that sustained viral suppression during monotherapy was more common among participants with a nadir CD4+ count above 200 cells/μL and no history of NNRTI use.

Short Cycles of Efavirenz-Based Therapy

Butler presented data on the use of short cycles of efavirenz-based antiretroviral therapy in HIV-infected children (Abstract 38LB). This study randomly assigned 199 participants to short cycles of (5 days on followed by 2 days off) or continuous efavirenz-based antiretroviral therapy. Participants were virally suppressed on efavirenz-based antiretroviral therapy, aged 8 years to 24 years, and had CD4+ counts greater than 350 cells/μL. Baseline characteristics of study participants included 47% female sex, a median age of 14 years, and median CD4+ count of 735 cells/μL. The median self-reported adherence rate was greater than 95% of scheduled drugs. The median number of days on drug were 72.8% and 99.8% for short cycles of therapy and continuous therapy, respectively.

Adherence to the randomized strategy was also confirmed by a Medication Event Monitoring System (MEMS) cap substudy and by analysis of mean corpuscular volume values for individuals taking zidovudine. The primary endpoint was a confirmed HIV-1 RNA level of 50 copies/mL or higher. Six participants (6.1%) in the short cycle–therapy group and 7 participants (7.3%) in the continuous-therapy group experienced the primary endpoint (difference, 1.2%; 95% CI, -4.9%-7.3%). This achieved protocol-defined noninferiority, suggesting the viability of this strategy for further study.

Discontinuing an Inactive nRTI

Llibre and colleagues investigated whether nRTIs can be safely discontinued in patients with extensive treatment histories for whom historical resistance testing predicts resistance to nRTIs (Abstract 553). The investigators randomly assigned 90 participants to discontinue an inactive nRTI or to maintain current nRTI-containing therapy. Among 45 participants assigned to discontinue an nRTI, 32 discontinued a single nRTI and 13 discontinued 2 nRTIs. Through week 48, 1 participant in each arm was not available for the week 48 analysis. Three participants who discontinued an nRTI had viremia at week 48 or had previously restarted an nRTI, and no viremia was observed

at week 48 in participants who maintained treatment with an nRTI. Noninferiority per the primary endpoint was not achieved in this small study.

Pharmacokinetic Considerations

Efavirenz Drug-Drug Interactions

Scarsi and colleagues investigated the drug-drug interactions between a long-acting subdermal implant of levonorgestrel and efavirenz (Abstract 85). The investigators enrolled HIV-infected women not taking antiretroviral therapy or taking an efavirenz-based regimen and compared levonorgestrel levels between the 2 groups. Efavirenz lowered the levonorgestrel concentrations by 48%. Moreover, 3 of 20 women taking an efavirenz-based regimen became pregnant, prompting closure of the study arm. The minimum effective levonorgestrel concentration required for contraception is likely much higher than previously thought.

Calderon and colleagues reported on the interaction of atovaquone with efavirenz or atazanavir/r compared with no antiretroviral therapy (Abstract 520). Coadministration of atovaquone 750 mg or 1500 mg twice daily with efavirenz led to a 47% and 44%, respectively, reduction in atovaquone exposure. No reduction was seen with atazanavir/r. This suggests that the currently recommended atovaquone dose for treatment or prophylaxis of *Pneumocystis jirovecii* pneumonia, 750 mg twice daily, may not be sufficient for patients receiving efavirenz.

Tenofovir and Moderate Renal Dysfunction

Cressey and colleagues investigated tenofovir diphosphate exposure in patients with moderate renal dysfunction (Abstract 511). Similar to patients with normal renal function, coadministration of TDF and lopinavir/r led to a higher exposure to tenofovir diphosphate than did NNRTI-based regimens; the area under the curve at 24 hours was 1.7 times higher, suggesting that TDF dose adjustment may be necessary when considering renal function and choice of regimen.

Fostemsavir

Fostemsavir (BMS-663068) is an investigational CD4+ attachment inhibitor that is currently in phase III clinical trials. The active form of fostemsavir, BMS-626529, is metabolized in part by cytochrome P450 3A4. Savant Landry and colleagues presented data on the interaction of fostemsavir with darunavir/r, etravirine, and darunavir plus etravirine in HIV-uninfected individuals (Abstract 523). Concentrations of BMS-626529 were decreased by approximately 50% with etravirine and were increased by 32% to 88% with darunavir/r or darunavir/r plus etravirine. No toxicities attributable to increased concentrations of BMS-626529 were noted. The investigators concluded that fostemsavir may likely be given with darunavir/r or darunavir/r plus etravirine without dose adjustment.

Pharmacokinetic Considerations During Pregnancy

Belissa and colleagues presented data on the pharmacokinetics and safety of raltegravir in 31 HIV-infected pregnant women (Abstract 891). They found that raltegravir concentrations were similar in these women compared with historical controls. They did not find maternal adverse events or adverse birth outcomes, and no infants became HIV-infected. These data support further study of InSTIs for pregnant women.

Two abstracts reported on etravirine concentrations during pregnancy (Abstracts 892 and 893). Both studies found that etravirine exposure was greater during pregnancy than postpartum, especially during the third trimester. Etravirine was generally well tolerated and both studies concluded that dose adjustments during pregnancy were not necessary. Mirochnick and colleagues investigated the use of rilpivirine during pregnancy (Abstract 894). Rilpivirine concentrations were similar in the second trimester, in the third trimester, and postpartum. The investigators concluded that no dose adjustment of rilpivirine is necessary during pregnancy.

The HIV Care Continuum as a Measure of Program Effectiveness

CROI 2015 expanded the previous emphasis on the HIV care continuum, sometimes called the HIV care cascade, as a metric of success in addressing the HIV epidemic,[3] with presentations from resource-rich and -limited settings, and further nuance regarding disparities at various stages along the care continuum.

Insights Into the HIV Care Continuum in the Industrialized World (Non–Resource-Limited Settings)

A number of studies examined the prevalence and predictors of progression along the care continuum in large cohorts in non–resource-limited settings. Retention in care data from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) study were examined by Rebeiro and colleagues, who found that having biyearly HIV clinic visits at least 90 days apart for 5 or more years was strongly associated with virologic suppression (prevalence ratio, 1.41; compared with individuals without continuous care; $P < .05$) (Abstract 996). The investigators also found statistically significant regional differences in virologic suppression, with the South faring worse (35.1%) and Canada faring best (66.2%). In this cohort, men who have sex with men (MSM) were statistically more likely to be virologically suppressed than injection drug users (IDUs) or heterosexuals with HIV transmission risk ($P <$ .01, for both), but blacks and Hispanics were statistically less likely than non-Hispanic whites to achieve virologic suppression ($P < .01$, for both). Buchacz and colleagues presented data from 9 US HIV treatment centers participating in the HIV Outpatient Study, examining the interaction between HIV transmission risk and race and ethnicity (Abstract 997). Non-Hispanic black

MSM had a statistically significantly lower rate of virologic suppression than non-Hispanic white or Hispanic MSM.

Looking in depth at a single US city, Torian and colleagues presented data on linkage to care and viral suppression in individuals aged 18 years or older who were newly diagnosed with HIV (from 2006-2013), using data from the New York City HIV Surveillance Registry (Abstract 99). The investigators found that linkage to care—defined as having a CD4+ cell count or plasma HIV-1 RNA level test within 3 months of HIV diagnosis—increased from 68% to 76% between 2006 and 2013 ($P < .0001$, for difference between 2 time points). Viral suppression (HIV-1 RNA < 400 copies/mL at 6 months and 12 months after diagnosis) also improved between 2006 and 2015, from 24% to 54% at 6 months and from 36% to 69% at 12 months ($P < .0001$, for both). It is encouraging that these outcomes improved across all age and CD4+ cell count strata over time. The investigators hypothesize that the 2010 New York State law requiring a standard of linkage to care within 3 months contributed to improvements in care engagement and that changes in national guidelines increasing the CD4+ cell count threshold for initiation of antiretroviral therapy contributed to improvements in viral suppression.

Significant sex- and race-related disparities in HIV treatment outcomes are apparent across numerous settings in the industrialized world.

These assertions are supported by the analysis of Braunstein and colleagues who found a statistically significant increase in median CD4+ cell count at time of HIV diagnosis (from 325 cells/μL in 2006 to 379 cells/μL in 2012), although there were consistent and statistically significant disparities among blacks, Hispanics, IDUs, heterosexuals, and women (Abstract 1001). Swain and colleagues examined the same cohort and found that 20% of individuals diagnosed with HIV in New York City between 2006 and 2010 who were still alive as of 2013 were out of care, defined by a lack of laboratory testing in 2012 (Abstract 1002). Men, MSM, and non-Hispanic blacks were statistically more likely to be out of care, and only 18% of those individuals returned to care in 2013.

Several groups examined the care continuum among HIV-infected women, highlighting the particular challenges of reaching this population in the United States. Ike and colleagues examined care engagement and viral suppression among women, using data from 17 US states and the District of Columbia reported to the US National HIV Surveillance System through December 2013 (Abstract 100). Linkage to care—defined as at least 1 CD4+ cell count or HIV-1 RNA level measurement within 3 months of diagnosis—occurred for at least 83% of women. Two measurements of retention in care were used: 1) at least 1 CD4+ cell count or HIV-1 RNA level measurement in 2011, and 2) 2 of the aforementioned laboratory tests occurring at least 3 months apart in 2011. Overall, retention in care rate was 67% by measurement 1 and 52% by measurement 2. However, statistically significantly higher retention rates were seen among Hispanic women and black women (69% and 59% vs 66% and 50% for the 2 measurements, respectively) than in white women (64% and 47%). Statistically significantly lower retention rates were seen among Asians (58% and 46%), Native Americans (47% and 33%), and Pacific Islanders (52% and 37%) than among whites (64% and 47%).

Hispanic women were statistically significantly more likely to be virologically suppressed (most recent HIV-1 RNA level, in 2011, < 200 copies/mL; 49%) than white women (47%), but black women were statistically significantly less likely to be virologically suppressed (42%) than white women. Younger women were less likely to be linked to care, engaged in care, or virologically suppressed, although the differences did not always reach statistical significance. Of concern, this sample represents 47% of all women in the United States diagnosed with HIV, yet almost half were not in regular care. Adams and colleagues examined retention in care and virologic suppression among postpartum women and found concerning drop offs in retention, with only 38% of women engaged in HIV care within 3 months of delivery and only 31% virologically suppressed at 1 year postpartum (Abstract 890). The results of these studies highlight the need to improve retention in HIV care for women, particularly young, black, or pregnant women.

Further Insights Into the HIV Care Continuum in Resource-Limited Settings

Maman and colleagues examined data on population viral load to determine how close communities are to achieving 90% viral suppression (per new Joint United Nations Programme on HIV/AIDS 90-90-90 guidelines[4]) in 3 settings in sub-Saharan Africa: Malawi, South Africa, and Kenya (Abstract 153). The investigators conducted multistage household-based surveys to recruit 19,005 adults aged 15 years to 59 years in sample households. HIV prevalence was 24.1% in Kenya, 17.0% in Malawi, and 25.2% in South Africa. Of individuals who were HIV infected, 40.0% in Kenya, 61.9% in Malawi, and 57.1% in South Africa had HIV-1 RNA levels of less than 1000 copies/mL. Of participants not taking antiretroviral therapy, men had statistically significantly higher HIV-1 RNA levels than women at all sites (82,503 copies/mL vs 29,885 copies/mL, respectively; $P < .01$), and this association remained statistically significant after adjusting for CD4+ cell count. These results are encouraging for those attempting to achieve the 90% target, but it is concerning that the majority of individuals with HIV-1 RNA levels of greater than 1000 copies/mL were undiagnosed.

Takuva and colleagues presented on HIV care engagement in South Africa using surveillance data from the National Health Laboratory Service, which captures all CD4+ cell counts and HIV-1 RNA levels measured in the public sector in the country (Abstract

154). CD4+ cell count measurement was used as a proxy for linkage to care in 2012, and measurement of HIV-1 RNA levels was used to determine the number of individuals taking antiretroviral therapy, as national policy does not include pre–antiretroviral therapy HIV-1 RNA levels. Using these definitions, 51% of the estimated 6,422,000 individuals with HIV infection in South Africa were linked to HIV care, 34% were taking antiretroviral therapy, and 25% had HIV-1 RNA levels of less than 400 copies/mL. Men were consistently less likely to engage in each phase of the care continuum, as were younger individuals ($P < .001$, for both). It is notable that the largest gap in the care continuum was in engagement in care, rather than initiation of antiretroviral therapy or virologic suppression of those taking antiretroviral therapy. The investigators highlighted the need for linkage-to-care efforts to improve outcomes for people living with HIV and to reduce the proportion of potentially infectious people living with HIV in South Africa.

Several national studies in Swaziland examined different stages in the care continuum. Ellman and colleagues used a nationally representative Swaziland incidence measurement survey to determine that 38% of adults with a positive HIV test result were unaware of their diagnosis; however, most of these individuals (83%) had been tested at least once previously for HIV (Abstract 1013). Men were at higher risk than women (odds ratio [OR], 2.48; 95% CI, 2.20, 2.80) for having unknown HIV serostatus.

In response to low uptake of antiretroviral therapy (35% of those eligible) in 2007, Swaziland implemented a "hub-and-spoke" system that developed linkages between treatment centers in cities with primary care clinics in more rural areas. Stable patients were referred by physicians in city hubs to nurses for care in primary care clinic "spokes." Patients were also able to initiate antiretroviral therapy in a primary care clinic during physician-led outreach visits from a hub. Antiretroviral coverage expanded to 84% based on antiretroviral therapy eligibility criteria at the time, and Auld and colleagues presented the results of their evaluation of the hub-and-spoke program (Abstract 155).

Home-based testing and counseling, decentralized HIV care that brings treatment centers closer to patients, and rapid availability of plasma HIV-1 RNA level testing are all associated with improvements along the HIV care continuum.

Sixteen of 31 existing hubs were sampled, using probability proportional to size sampling and simple random sampling for individual patients. Spoke-initiated patients were statistically significantly more likely to be women and unmarried than those who were maintained in care in the hub or who were down-referred from hub care to spoke care after initiation of antiretroviral therapy. In Cox proportional hazard models adjusted for demographic and clinical variables, neither down-referral nor spoke-level initiation of antiretroviral therapy was associated with mortality but both were associated with statistically significant reductions in loss to follow-up and attrition (range of reductions, 50%-62%). This national-level evaluation of a decentralization program for antiretroviral therapy is encouraging and implies that this methodology can be used to increase access to antiretroviral therapy without compromising some basic care metrics.

Cross and colleagues examined unscheduled treatment interruptions in 40,632 patients receiving antiretroviral therapy at 33 Médicins Sans Frontières sites across 11 countries in Africa and Asia between 2003 and 2013 (Abstract 1011). Overall, 25% of patients had more than 1 treatment interruption of more than 90 days, with women and younger patients (aged <20 years) being at statistically higher risk for interruption than men and those aged 20 years or older, respectively. Interruptions were also statistically significantly associated with having a CD4+ count of less than 200 cells/μL and WHO stage 4 HIV disease at initiation of antiretroviral therapy.

Solomon and colleagues presented data from a multisite study of engagement in care among 12,022 HIV-infected MSM and 14,481 HIV-infected IDUs in India (Abstract 1016). Eighty percent were linked to care and 59% of those unlinked had been diagnosed within the past year. Linkage to care among MSM and IDUs was statistically significantly associated with having received help with a medical appointment or transportation at time of diagnosis (OR, 10.0; 95% CI, 5.6, 18.2) and with disclosure of HIV serostatus to 1 or more people (OR, 2.8; 95% CI, 2.4, 6.1).

Rachlis and colleagues used data from the International Epidemiologic Databases to Evaluate AIDS (IeDEA) in East Africa to examine facility-level factors associated with retention in care among 88,152 patients in 29 clinics in Kenya, Tanzania, and Uganda (Abstract 1072). The investigators found that loss to follow-up—defined as no clinic visits for 12 months for individuals not yet receiving antiretroviral therapy and no clinic visits for 6 months for those taking antiretroviral therapy—was more common in clinics where HIV-1 RNA level testing took longer than 14 days (hazard ratio [HR], 1.30; 95% CI, 1.21, 1.41) and where CD4+ cell counts were not available on site (HR, 1.23; 95% CI, 1.09, 1.38). Clinics that were open more than 4 days a week were less likely to experience loss to follow-up (HR, 0.73; 95% CI, 0.61, 0.89).

Antiretroviral Therapy Scale-Up and Treatment in Resource-Limited Settings

Ambassador Deborah Birx outlined the strategy for the President's Emergency Plan for AIDS Relief (PEPFAR) 3.0, in which the program builds upon its past success to deploy resources and support (Abstract 96). She emphasized the need for county- and site-level data to direct interventions to areas where they will have high impact, using the example of Nairobi, Kenya, where adjacent perinatal HIV testing sites have widely different HIV

prevalence. Efforts should focus on sites with higher prevalence and rural areas where HIV testing and treatment can be concentrated along routes where HIV incidence is higher. Birx also discussed threats to future success, highlighting the recent increase in HIV incidence and prevalence in Uganda, the anticipated increase in a susceptible population in sub-Saharan Africa (the large school-age population becoming older), and, perhaps most importantly, the threat to HIV funding overall. Birx suggested that these threats might be ameliorated by clear demonstrations of the positive health and economic impacts of interventions.

Life Expectancy and Mortality in Resource-Limited Settings

Dramatic improvements in overall adult life expectancy in the past 10 years in sub-Saharan Africa have been attributed to the scale-up of antiretroviral therapy in this highly impacted region. Reniers and colleagues used data from the Analysing Longitudinal Population-Based HIV/AIDS Data on Africa (ALPHA) network, a chain of demographic surveillance sites in Uganda, Tanzania, Kenya, Malawi, and South Africa that conducts repeated community-based HIV testing, to estimate population-based mortality based on HIV serostatus (Abstract 161). Nonparametric analysis determined life expectancy gains after the introduction of antiretroviral therapy and the life expectancy deficit, which is the life expectancy of someone uninfected with HIV subtracted from the overall life expectancy, giving an estimate of the continued impact of HIV on life expectancy for the region. The investigators found some life expectancy gains prior to antiretroviral therapy scale-up, but gross life expectancy gains once antiretroviral therapy was available were between 6 years and 15 years and were statistically significantly greater for women than men. The life expectancy deficit caused by HIV infection was less than 5 years in most sites but was more than 10 years in Kisumu, Kenya, and in a site in South Africa, in which women were more highly impacted. Investigators also examined whether gains in life expectancy could actually be attributed to antiretroviral therapy by estimating the counterfactual life expectancy based on trends pre–antiretroviral therapy. Overall, net gains in life expectancy realized with antiretroviral therapy were greater for South African sites because of the baseline decreased life expectancy in that region. The investigators highlighted that examination of gross life expectancy gains without adjusting for overall trends in life expectancy would underestimate the impact of antiretroviral therapy in South Africa and overestimate it in much of Eastern Africa, but that the burden of HIV on adult mortality remains important at 5 years to 10 years.

Taking a more global look at post-seroconversion survival, Mangal presented data on CD4+ cell count trajectories and mortality from cohorts of HIV-infected individuals who seroconverted in North America, Europe, Africa, and Asia (Abstract 97). Investigators estimated CD4+ cell count decline and survival across cohorts, adjusting for age, sex, and region for patients not yet receiving antiretroviral therapy using a continuous-time Markov model. The investigators found that 50% to 55% of men seroconverted with a CD4+ cell count of more than 500 cells/μL and that median adjusted survival for men aged 20 years at seroconversion was statistically significantly shorter for men in Asia (6.9 years; 95% CI, 6.2, 8.1) than for those in Africa (10.8 years; 95% CI, 10.4, 11.3) or in Europe and North America (12.3 years; 95% CI, 10.7, 12.8). CD4+ cell count decline and mortality increased with increasing age and were similar in African, European, and North American cohorts, but were more rapid in Asian cohorts.

Pierre and colleagues examined the characteristics of HIV-infected individuals in Haiti who had survived for 10 years after initiation of antiretroviral therapy (2003-2013) despite numerous challenges, including an earthquake in 2007, a subsequent cholera epidemic, and political instability (Abstract 156). Investigators conducted a retrospective study of routinely collected clinical data from 910 patients initiating antiretroviral therapy between 2003 and 2004. Home visits were used to trace patients who were lost to follow-up for more than 180 days. Fifty-three percent of the 910 patients were alive 10 years after initiation of antiretroviral therapy, 27% had died, and 13% remained lost to follow-up. Being older than 50 years, being underweight, having a missing CD4+ cell count or a having CD4+ count of less than 50 cells/μL, and having WHO stage 3 or 4 HIV disease were associated with death within the first 6 months of antiretroviral therapy.

Dramatic improvements in life expectancy have been achieved for people with HIV infection in resource-limited settings, but the negative consequences of long-term HIV infection are still apparent even after 10 years of antiretroviral therapy.

Younger age (13 years-24 years) was associated with loss to follow-up within the first 6 months of antiretroviral therapy and after. Among the 482 individuals who survived for 10 years, 38% had evidence of a noncommunicable disease, including 109 individuals with cardiovascular disease and 67 with chronic obstructive pulmonary disease.

Examinations of the Cost-Effectiveness and Financial Implications of Antiretroviral Therapy Scale-Up

Several interesting examinations of the cost-effectiveness of antiretroviral therapy scale-up and specific scale-up strategies in resource-limited settings took advantage of the 10 years of data collected since programmatic rollout of antiretroviral therapy began. Luz and colleagues estimated years of life saved by the Brazilian national HIV treatment program between 1997 and 2014, using a microsimulation model (Abstract 1119). Per capita survival

benefit in years conferred by antiretroviral therapy increased from 7.0 in 1997 to 18.9 in 2014, largely driven by increases in CD4+ cell count at initiation of antiretroviral therapy. The cumulative survival benefit of antiretroviral therapy to the 618,561 individuals who started treatment between 1997 and 2014 was estimated at 1,476,638 life-years in 2015, highlighting the power of successful country-wide initiatives.

Smith and colleagues examined the impact of home-based HIV testing and counseling with facilitated linkage to care on programmatic cost and cost-effectiveness ratios in rural KwaZulu-Natal, South Africa, through an individual-based mathematical model testing the impact on HIV incidence and disease-adjusted life-years (Abstract 1111). Although home testing and counseling are more expensive, the estimated 91% testing coverage and 80% antiretroviral therapy uptake at 6 months achieved by these programs has the potential to reduce HIV incidence by 36%. The incremental cost-effectiveness ratio (ICER) per disease-adjusted life-year averted was less than 20% of the South African gross domestic product per capita, a threshold for appropriate cost-effectiveness, at all CD4+ cell count thresholds for the initiation of antiretroviral therapy. Importantly, overall costs were driven primarily by the cost of antiretroviral therapy rather than costs of the testing and counseling strategy, and reducing costs to the global target of $200 per person per year would reduce the ICER by 36% to 76%. The investigators pointed out that the most effective way to decrease costs is by averting infection, as each infection leads to a fixed cost in lifelong antiretroviral therapy.

The impact of the cost of antiretroviral therapy on the cost of overall HIV treatment in resource-limited settings was evident in the work of Onyekwena and colleagues of the Global Fund to Fight AIDS, Tuberculosis, and Malaria, who estimated the impact of expanded antiretroviral therapy proposed in the 2013 WHO HIV treatment guidelines (Abstract 1114).[2] The investigators found that medication costs alone would take up 41% of total current allocations to the Global Fund for all HIV services for the 32 countries receiving Global Fund support included in the model. However, this did not take into account the additional facility, monitoring, and adherence support needed to provide antiretroviral therapy to an estimated 2.8 million additional people who meet eligibility criteria under the new guidelines. Additional funding is needed to support comprehensive services, if they are to be delivered in accordance with the new guidelines.

The price of antiretroviral therapy itself is the key driver of cost and cost-effectiveness of HIV treatment in resource-limited settings.

Bor and colleagues approached the same problem, the impact of antiretroviral therapy at higher CD4+ cell count thresholds, using a quasi-experimental regression discontinuity model to examine survival of individuals presenting for care with CD4+ counts just above or just below the 200 cells/μL threshold (Abstract 1110). Using data on 4391 patients in South Africa seeking care between 2007 and 2011, the investigators found that immediate initiation of antiretroviral therapy, as opposed to delayed initiation, for those with CD4+ counts near 200 cells/μL led to reduced mortality (HR, 0.65; 95% CI, 0.45, 0.94). Earlier initiation also saved 0.18 life-years within 5 years, at a cost of US $1967 per life-year saved. These data support the immediate initiation of antiretroviral therapy for individuals in South Africa with CD4+ counts near 200 cells/μL but do not address the impact of higher thresholds, such as those in the 2013 WHO treatment guidelines.[2]

Hontelez and colleagues from the Africa Centre in Kwazulu-Natal, South Africa, used data beginning in 2000 from a continuous, full-population cohort of more than 100,000 individuals to determine the effects of antiretroviral therapy scale-up on health care utilization among HIV-infected and -uninfected individuals (Abstract 159). When the investigators examined trends in health care utilization for 33,563 people observed over 57,821 person-years, there was a statistically significant increase in utilization of public sector health care and a statistically significant decrease in hospitalizations, and utilization of private sector care decreased for both HIV-infected and -uninfected individuals, even after adjustment for age, sex, and location of residence. These data imply that the scale-up of antiretroviral therapy has increased hospital capacity in the region. The investigators speculated that the efficiency of health care delivery is improved and that out-of-pocket expenditures for health care are diminished because public care is less expensive than private care. The fact that these potential benefits apply to HIV-infected as well as -uninfected individuals is encouraging; however, further research is needed to understand the mechanism of these shifts in health care delivery and cost-effectiveness.

Treatment Strategies and Outcomes for Children and Adolescents in Resource-Limited Settings

Gibb gave an excellent overview of current challenges in scale-up and treatment of HIV-infected children (Session PL-1; Abstract 78). Implementation of the 2013 WHO HIV treatment guidelines,[2] which recommend immediate antiretroviral treatment for all HIV-infected children under age 5 years, creates a new and unmet need for antiretroviral therapy in these children. Some countries, such as Uganda, have extended this recommendation to include immediate antiretroviral treatment for all children under age 15 years, and examinations of the impact of this change will be essential to direct future guidelines. Gibb outlined several challenges to further expanding access of antiretroviral therapy to children, including the paucity of pediatric formulations of antiretroviral therapy. Most children remain on an initial regimen of nevirapine, abacavir, and lamivudine. There are

few second-line options, as protease inhibitor (PI)-based regimens are expensive and most formulations are unpalatable for children and integrase inhibitor–based regimens are currently unavailable. Lack of appropriate laboratory monitoring for children is also a challenge in many resource-limited settings, as is maintenance of adherence and engagement in care for perinatally infected children as they reach adolescence.

Payne and colleagues examined the impact of early antiretroviral therapy on the proviral reservoir in children, using data from the CHER (Children with HIV Early Antiretroviral Therapy) trial (Abstract 35). Children enrolled in the trial, which demonstrated a dramatic 76% ($P = .0002$) reduction in mortality among those receiving 40 weeks of early antiretroviral therapy, had HIV-1 proviral DNA measured by quantitative polymerase chain reaction (PCR) from DNA extracted from peripheral blood mononuclear cells (PBMCs) at 40 weeks, 96 weeks, and 248 weeks into the trial. The investigators found that HIV-1 proviral DNA was statistically significantly lower in children starting early antiretroviral therapy (median, 27 provirus copies/105 PBMCs; interquartile range [IQR] 8-21) than in those deferring treatment until they met clinical thresholds (median, 100 provirus copies/105 PBMCs; IQR 42202; $P < .0001$, for the difference). However, proviral DNA levels increased after treatment interruption, and at the end of the trial (248 weeks) there was no statistically significant difference in proviral DNA levels between the early and deferred-treatment arms.

Challenges to antiretroviral treatment of HIV-infected children include the scarcity of palatable pediatric formulations, particularly of second- and third-line regimens, and the unknown long-term toxic effects of antiretroviral therapy.

Of note, all children whose antiretroviral treatment was interrupted had resurgence of HIV-1 RNA levels. These data could imply that the benefits of early antiretroviral treatment, with regard to proviral burden, are lost after treatment interruption.

A cohort of children from the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network P1060 study followed up for 5 years while taking nevirapine- or lopinavir/r-based regimens was examined by Barlow-Mosha and colleagues to determine the long-term outcomes of these regimens in children (Abstract 36). There were similar improvements in CD4+ cell counts and weight and height *z* scores in the groups. However, only 52% of children remained on nevirapine, as opposed to 84% with lopinavir/r, and there was an increased risk for virologic failure or death in the group receiving nevirapine compared with the group receiving lopinavir/r (adjusted HR, 1.9; 95% CI, 1.4, 2.7). The investigators concluded that these findings support the current WHO recommendations for use of lopinavir/r as an initial regimen for children, although new formulations and further information on the long-term metabolic effects of lopinavir/r in children are needed.

Prevention of Mother-to-Child Transmission

Pregnancy and Prevention of Mother-to-Child Transmission

As part of a plenary session, Gibb described a 60% drop in new pediatric HIV infections globally since 2000, along with the increase in prevention of mother-to-child transmission (PMTCT) coverage and shifts toward adoption of Option B and B+ programs (Session PL-1; Abstract 78). In contrast to Option A (only provide lifelong antiretroviral treatment for women who meet clinical or immunologic criteria, and use antenatal zidovudine with single-dose nevirapine at delivery followed by an nRTI tail for others), Options B and B+ provide treatment for all HIV-infected pregnant women regardless of their clinical or immunologic status. Under Option B, women who do not meet clinical or immunologic criteria for continuation of antiretroviral therapy discontinue treatment after cessation of breastfeeding, whereas under Option B+ all women remain on lifelong antiretroviral therapy regardless of whether they are breastfeeding. In Options B and B+, infants receive 4 weeks to 6 weeks of nevirapine or zidovudine.

With the adoption of Option B and B+, PMTCT coverage has increased to 70%, contributing to the 60% drop in new pediatric HIV infections since 2000 mentioned above. However, PMTCT coverage has been highly variable across countries, and there are large fall-offs in the care cascade, especially postpartum. In 2013, there were still approximately 240,000 new HIV infections attributable to MTCT.

Gibb highlighted findings from the PROMISE (Promoting Maternal-Infant Survival Everywhere) trial, which were also presented by Fowler and colleagues (Session O-2; Abstract 31LB). PROMISE is an ongoing randomized controlled trial in 5 African countries and India comparing the benefits of triple antiretroviral therapy with the benefits of zidovudine monotherapy in HIV-infected pregnant women with CD4+ counts higher than 350 cells/μL. Safety and efficacy results were presented from antepartum through 14 days postpartum. HIV-infected pregnant women were randomly assigned to receive zidovudine with a tenofovir and emtricitabine tail (arm A), zidovudine, lamivudine, and lopinavir/r (arm B), or tenofovir, emtricitabine, and lopinavir/r (arm C).

The analysis included 3529 pregnant women, with 3234 live births. There were statistically significant differences in MTCT rates by 14 days of age in the 2 triple-therapy arms compared with zidovudine monotherapy (0.5% vs. 1.8%, respectively; risk difference, -1.28%; 95% CI, -2.11%, -0.44%). However, there were also significant differences in infant deaths by age 14 days (0.6% vs. 4.4% in arms B and C, respectively; $P = .001$); deaths primarily occurred in infants delivered before 34 weeks of gestation (2.6% vs. 6% in arms B and C, respectively; $P = .04$). These infant outcomes were not statistically significant when comparing arm

B or C with arm A (monotherapy arm). The investigators reported more grade 2 to 4 adverse events in arms B and C and more moderate but not severe pregnancy outcomes, including birthweight below 2500 g or birth before 37 weeks. The investigators concluded that these results support the 2013 WHO recommendations to use triple antiretroviral therapy for pregnant women,[2] to safely achieve the lowest risk of HIV transmission and to urge further exploration of the unexpected increased risk of infant death during triple therapy that includes tenofovir and emtricitabine.

The PROMISE Trial showed reduced rates of MTCT for women who received triple antiretroviral therapy compared with women who received zidovudine monotherapy. There is an unexpectedly higher rate of infant death with tenofovir-containing triple therapy than with zidovudine-containing triple therapy.

Viral Suppression During Pregnancy and Perinatal Transmission:

Maman and colleagues presented viral load data on pregnant and breastfeeding women from a cross-sectional population survey and on HIV-testing from Malawi under Option B+, South Africa under Option B, and Kenya under Option A (Abstract 32). If women had a negative HIV test result, a nucleic acid amplification test was done. In sites where Option B or B+ had been implemented, there was less loss to follow-up along the care cascade. Of women surveyed who were HIV-infected and pregnant or breastfeeding in Malawi, South Africa, and Kenya, 80%, 65%, and 50%, respectively, were linked to care; 75%, 55%, and 25%, respectively, were taking antiretroviral therapy; and 72%, 63%, and 22%, respectively, had an HIV RNA level of less than 1000 copies/mL. Of breastfeeding women with an HIV RNA level greater than 1000 copies/mL, 58.6% (95% CI, 52%-65%) did not know their HIV serostatus at the time of the survey, which was similar across the 3 sites. Overall, 4.1% of breastfeeding women were infected with HIV during pregnancy or while they were breastfeeding (6.5% in Kenya, 4.3% in South Africa, and 1.9% in Malawi), with these newly diagnosed infections accounting for 37.5% of HIV-infected, breastfeeding women with HIV RNA levels above 1000 copies/mL. Using incidence assays, HIV-incidence among women aged 15 years to 29 years was 3.8 per 100 person-years in Kenya, 3.2 per 100 person-years in South Africa, and 0.9 per 100 person-years in Malawi. The investigators concluded that broader approaches to PMTCT are needed and that investments in early infant HIV diagnosis remain important.

A themed discussion (Session TD-T) focused on viral suppression during pregnancy and risks of perinatal transmission. Mandelbrot led the discussion and presented data from the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS) French Perinatal Cohort of 8075 HIV-infected women, showing that risk of MTCT increased with higher maternal viral load at delivery (Abstract 867). Risk also increased incrementally the later antiretroviral therapy was started during pregnancy, independent of viral suppression at delivery. There were no cases of MTCT among the 2651 women who were virally suppressed before conception.

Ellington and colleagues from the BAN (Breastfeeding, Antiretrovirals, and Nutrition) study evaluated risk factors for MTCT and found that a viral load of greater than 10,000 copies/mL increased odds of MTCT (OR, 17.7; 95% CI, 2.5-128) (Abstract 868). Food shortage, history of herpes simplex virus infection, and a reported sexually transmitted infection in the last 12 months were also independent predictors of MTCT. Powis and colleagues from Botswana (Abstract 870) analyzed MTCT rates before and after the rollout of Option B in an observational cohort that included 2527 infants. MTCT rates with Option A and Option B were 1.6% and 0.7%, respectively ($P = .04$), which were comparable to the rates in the PROMISE study. Risk factors for transmission included being on triple antiretroviral therapy for less than 4 weeks and absence of viral suppression (HIV RNA level <40 copies/mL) at delivery.

Sripan and colleagues presented results from a modeling analysis based on 1655 viral load results from 702 HIV-infected pregnant women from the Thai PHPT-5 (Program for HIV Prevention and Treatment–5) study in which women received zidovudine alone, zidovudine and lopinavir/r, or triple therapy with zidovudine, lamivudine, and lopinavir/r (Abstract 863). Modeling was based on prediction of viral load at delivery as a function of treatment duration. The triple-therapy arm reached viral suppression (HIV RNA level <40 copies/mL) at 4.4 weeks, an estimated 3 weeks faster than the arm that received zidovudine and lopinavir/r. Monotherapy with zidovudine never resulted in viral suppression

Myer and colleagues presented results from a prospective cohort of 624 HIV-infected pregnant women in South Africa initiating antiretroviral therapy with viral load measurements before initiation of antiretroviral therapy, 2 weeks to 4 weeks after the initiation of antiretroviral therapy, late in the third trimester, and before delivery (Abstract 864). Most women achieved an HIV RNA level below 1000 copies/mL within 4 weeks of initiation of therapy with tenofovir, emtricitabine, and efavirenz, and an HIV RNA level below 50 copies/mL by 14 weeks. By time of delivery, 73% of women had achieved an HIV RNA level of less than 50 copies/mL, but results were heavily influenced by viral load before the initiation of antiretroviral therapy. On multivariable analysis, viral load before initiation of antiretroviral therapy and gestational age at initiation of antiretroviral therapy were the main determinants of viral suppression by the time of delivery. There was a rapid early drop in HIV RNA level to below 1000 copies/mL, but a large proportion of women do not reach less than 50 copies/mL by delivery.

Bobrow and colleagues presented data from the Kabeho (Kigali Antiretroviral and Breastfeeding Assessment for the Elimination of HIV) study, a

prospective observational cohort in Rwanda, in the context of Option B+ (Abstract 865). Most of the 608 women in the study were taking a regimen of tenofovir, lamivudine, and efavirenz and had viral load testing in their third trimester or within 2 weeks postpartum. Half of the women were virally suppressed (HIV RNA level < 20 copies/mL) at study enrollment. Risk factors for detectable viral load were lack of education, adverse drug effects reported in the last month, and taking antiretroviral therapy for less than 4 months. Sixty-six percent of participants achieved viral suppression by 4 months of antiretroviral therapy. The subsequent discussion following these presentations focused on the potential of using more potent regimens, such as those containing nevirapine or integrase inhibitors, for which there are few data for pregnant women, to rapidly suppress the viral load.

These studies highlight the high rates of detectable viral load among pregnant women at delivery, the importance of starting antiretroviral therapy early, and the significance of viral load suppression for preventing MTCT. Ongoing MTCT data are being collected in several of these studies.

Adherence and Retention in Care

Several studies evaluated adherence and retention in care in the context of the rollout of Option B+. Sebastian and colleagues examined data from 22,300 mother-and-child pairs enrolled in care before the adoption of Option B+ and 25,500 mother-and-child pairs enrolled in care after the adoption of Option B+ in Mozambique (Abstract 873). The investigators found that a higher proportion of HIV-infected pregnant women were receiving antiretroviral therapy under Option B+ (37% vs. 94%, respectively; $P = .05$). However, less than half of HIV-exposed infants in each group had PCR testing done; of those in whom a PCR test was performed, 6% pre-B+ implementation and 4% in post-B+ implementation had positive results ($P = .03$).

Domercant and colleagues described retention rates under Option B+ among 1365 HIV-infected pregnant women compared with HIV-infected men and women who were not pregnant (Abstract 875). Six months after initiation of antiretroviral therapy, retention rates for women under Option B+ were lower than for men and nonpregnant women (74% vs. 81%; adjusted relative risk [RR], 0.91; $P < .001$).

Phillips and colleagues evaluated adverse events during the first 2 months of antiretroviral therapy with tenofovir, emtricitabine, and efavirenz in a cohort of HIV-infected pregnant women (Abstract 888); 73% experienced central nervous system adverse effects, 66% experienced gastrointestinal adverse effects, 19% experienced dermatologic adverse effects, and 63% experienced systemic adverse effects. In a multivariable analysis adjusted for age, duration of antiretroviral therapy, and timing of HIV diagnosis, only dermatologic adverse effects were associated with self-reported missed doses (OR, 2.17; 95% CI, 1.2-3.96).

Davis and colleagues reported on a case-control study of 31 HIV-infected mothers taking and 232 mothers not taking antiretroviral therapy, using data from the BAN study (Abstract 886). Better adherence was associated with a lower detectable viral load in breast milk (HIV RNA level < 40 copies/mL), and having detectable HIV RNA in breast milk was associated with MTCT (adjusted HR, 7.4) from 2 weeks to 28 weeks postpartum. These results illustrate the importance of adherence and retention in care postpartum.

Adams and colleagues reported on the postpartum care continuum among 591 HIV-infected pregnant women in Philadelphia, Pennsylvania, using retrospective cohort surveillance data. At 1 year postpartum, 39% of women remained in care with 31% virally suppressed, and at 2 years postpartum, 25% remained in care with 33% virally suppressed (Abstract 890). Women who engaged in HIV care within 3 months of delivery were more likely to remain in care and be virally suppressed at 1 year and 2 years postpartum. These studies highlight some of the challenges to retention in care, adherence to antiretroviral therapy, and viral suppression.

PMTCT-Related Drug Resistance

Hoffman and colleagues, as part of the TSHEPISO study, also reported on viral suppression postpartum in a cohort of 103 South African, HIV-infected, pregnant women taking antiretroviral therapy (83% efavirenz based and 9.7% nevirapine based) with 12 months of follow-up after delivery (Abstract 907). Of 103 women included in the analysis, 43 (42%) were being treated for tuberculosis at the time of enrollment. The analysis compared viral suppression rates during pregnancy with viral suppression rates 12 months after birth. Eighty-seven percent of the women were virally suppressed while pregnant, but only 71% of these women were virally suppressed 12 months postpartum. Of those who did not have viral suppression at 12 months, 46% had NNRTI-associated resistance mutations and 15% had the M184V mutation, and there was concern that depression contributed to poor adherence and limited retention in care.

Ledwaba and colleagues conducted deep sequencing of virus on 201 plasma samples from pregnant women who had transmitted HIV subtype C to their infants (Abstract 908). Forty-six percent (53/115) of those receiving Option A during pregnancy had resistant virus, 80% (12/15) receiving Option B had resistant virus, and 18% (12/65) of those who reported that they had not received antiretroviral therapy for PMTCT had resistant virus. Of those with resistant virus, 34% had virus resistant to NNRTIs. These results suggest suboptimal adherence or regimen failure during PMTCT treatment.

Nelson and colleagues evaluated resistance mutations in infants with HIV-infected mothers as part of the BAN study, in which mother-and-infant pairs were given single-dose nevirapine, the mothers were given a tail of lamivudine and zidovudine at birth, and then the pairs were randomly assigned to 1 of 3 possible arms during

the breastfeeding period: the infants received nevirapine, the mothers received combination antiretroviral therapy, or neither the mother nor child received medication (Abstract 909). The investigators reported that nevirapine prophylaxis in infants reduced the risk of HIV but that 50% of infants had resistance to nevirapine if they became infected, with K103N being the most common mutation. These studies suggest high rates of emergence of resistance during PMTCT, in particular to NNRTIs, with K103N being a common mutation.

Health Outcomes of HIV-Exposed Infants

Zash and colleagues reported birth outcome data associated with tenofovir, emtricitabine, and efavirenz used as PMTCT in Botswana in a retrospective chart review of 5247 HIV-infected pregnant women (Abstract 878). Of these women, 28% were taking tenofovir, emtricitabine, and efavirenz, 15% were taking other triple-drug anti-retroviral regimens, and 56% were taking zidovudine alone. Overall, 18% of infants were small for their gestational age, 21% of women delivered preterm, and 3% of women had stillbirths. The women taking tenofovir, emtricitabine, and efavirenz had fewer infants who were small for gestational age (adjusted OR, 0.6; 95% CI, 0.4, 0.8) than women who were taking other triple-therapy regimens or zidovudine. No differences were seen between the groups in terms of preterm delivery. The investigators concluded that although adverse birth outcomes remained high, tenofovir, emtricitabine, and efavirenz appears to be as safe as other antiretroviral regimens and was associated with fewer small for gestational age infants.

Liotta and colleagues also evaluated tenofovir, emtricitabine, and efavirenz exposure in Malawian infants under Option B+, evaluating growth and bone markers (Abstract 879). In 103 infants, biomarkers for bone formation and resorption were at levels similar to reference pediatric standards.

Guerra and colleagues reported data from the PHACS (Pediatric HIV/AIDS Cohort Study) study, evaluating the echocardiograms of 174 youths who were HIV-uninfected but had been exposed to HIV. Of these youths, 18 had not been exposed to antiretroviral drugs, 89 had some antiretroviral drug exposure, and 67 had been exposed to combination antiretroviral therapy (Abstract 882). Overall left ventricular systolic function was similar across groups, but more subtle findings of mitral late diastolic inflow velocities and left ventricle mass-to-volume *z* scores were statistically significantly lower in those who had been exposed to antiretroviral therapy than in those who had not, suggesting that continued cardiac monitoring may be needed in this population.

Pharmacokinetics and Antiretroviral Safety During Pregnancy

Two abstracts reported pharmacokinetic and safety data on etravirine 200 mg twice daily during pregnancy. Best and colleagues reported pharmacokinetic data from 5, 13, and 9 women who were in the second trimester, third trimester, and postpartum, respectively (Abstract 892). Etravirine exposure was higher in women in their third trimester than in postpartum women and nonpregnant controls. Four infants had grade 3 or 4 lab abnormalities. Ramgopal and colleagues also reported higher exposures to etravirine during pregnancy than postpartum (Abstract 893). Of 15 women in the study, 12 had adverse events, with 4 serious adverse events thought to be unrelated to etravirine use. No one discontinued etravirine owing to adverse events.

Mirochnick and colleagues examined the adverse events associated with and the pharmacokinetics of rilpivirine in 32 pregnant women (Abstract 894). Exposure was reduced during pregnancy but levels were still above targets at standard adult doses. There were 4 maternal and 4 infant adverse events.

Considering InSTI use during pregnancy, Belissa and colleagues described a study of 23 HIV-infected women receiving raltegravir who initiated treatment at least 2 weeks before delivery (Abstract 891). Median duration of raltegravir use was 8.1 months, with an IQR of 2.6 months to 67.1 months. The investigators reported large interpatient variability. However, raltegravir plasma concentrations were similar to historical data in nonpregnant women at the same dose (400 mg twice daily), with favorable placental transfer and accumulation in amniotic fluid. Four infants were delivered at or before 37 weeks gestation; no other infant adverse events were reported, and 1 woman stopped treatment because of hepatic function abnormalities. All women reached viral suppression (HIV RNA level <400 copies/mL) by delivery except for 1 late presenter who had an HIV RNA level of 500 copies/mL, and 74% had HIV RNA levels below 50 copies/mL at time of delivery.

Maternal/Infant MTCT Mechanisms

Woods and colleagues reported data on 156 HIV-unexposed infants in South Africa who were either exclusively breastfed or received mixed feeding (Abstract 897). Oral swab, blood, and stool samples were collected. Infants who received mixed feeding had a higher percentage of oral HIV-target cells ($P = .009$), higher levels of activated CD4+ cells ($P = .002$) in blood, and an increased proportion of Ruminococcus in stool ($P = .02$). These data suggest that increased HIV susceptibility of infants receiving mixed feeding may be mediated by an increase in HIV-susceptible cells in the oral mucosa and systemic circulation.

Antiretroviral therapy has been associated with pregnancy complications, including low birth weight and preterm delivery potentially owing to low progesterone levels. Papp and Serghides examined pathways of progesterone synthesis in placental tissues and the effects of PI-based antiretroviral therapy in 33 HIV-infected women and 14 HIV-uninfected women (Abstract 902). The investigators described low prolactin levels and increased expression of 20α-hydroxysteroid, an enzyme

that inactivates progesterone, in HIV-infected women exposed to antiretroviral therapy. Inhibition of 20α-hydroxysteroid led to recovery of progesterone levels, and increased prolactin levels in placental cells decreased 20α-hydroxysteroid expression.

Fouda and colleagues reported results from the Pediatric AIDS Clinical Trials Group (PACTG) 230 study of antibody responses in 49 HIV-exposed infants immunized with 4 doses of recombinant glycoprotein (gp)120 vaccine and 18 HIV-exposed infants who received a placebo (Abstract 905). The vaccine elicited a broad response in immunoglobulin G (IgG), including potentially protective IgG1 and IgG3 anti-V1V2. Avidity of the vaccine-elicited anti-V1V2 was similar in the infants to that in their chronically HIV-infected mothers.

Early Infant Diagnosis

Gibb also discussed the care cascade in Malawi, the first country to adopt Option B+ (Abstract 78). Although 97% of pregnant women had at least 1 antenatal clinic visit, there were steep drop-offs along the care cascade. Of the pregnant women presenting to antenatal care, 15% had not been tested for HIV. Of those who initiated treatment under Option B+, 23% were lost to follow-up in less than 6 months and 30% were lost to follow-up by 24 months. Additionally, of the 2850 children who started antiretroviral therapy in Malawi from April 2014 to June 2014, only 3.7% had their infection identified by DNA PCR testing.

Gibb also presented 2014 data from Kenya illustrating the challenges of early infant diagnosis. Only 6% of HIV-infected children in Kenya were diagnosed in the context of PMTCT, and 21% and 31% of HIV-infected children were diagnosed as outpatients or in the pediatric ward, respectively. These data highlight the importance of provider-initiated HIV testing in addition to that in the setting of PMTCT.

Njuguna and colleagues reported gaps in HIV prevention and diagnosis among HIV-infected, antiretroviral treatment–naive children aged 0 years to 12 years in Kenya who were hospitalized at time of enrollment in the study, representing HIV-infected children not linked to care (Abstract 911). Twenty-five percent of the mothers did not have an HIV test during antenatal care, 42% had a negative HIV test result during antenatal care but became HIV infected in late pregnancy or postpartum, and 12.4% of the children were not diagnosed in early infancy and did not undergo clinician-initiated testing and counseling. The investigators concluded that these results accounted for the bulk of gaps in missed diagnoses and linkage to care in Kenya and suggested a need for repeat HIV testing in late pregnancy and postpartum.

Based on household survey data from Kenya, Malawi, and South Africa, a statistically significant portion of women were found to be HIV-infected during pregnancy or breastfeeding, supporting the need for repeat HIV testing until the end of the breastfeeding period.

King presented data from the BAN study evaluating HIV detection in exposed infants (Session O-2; Abstract 33). Following a single dose of nevirapine and a tail of lamivudine and zidovudine, the BAN study randomly assigned infants to receive 28 weeks of nevirapine, to have their mothers receive combination antiretroviral therapy, or to receive a single dose of nevirapine (control) during the breastfeeding period. After cessation of breastfeeding and antiretroviral therapy, there were 28 infants with HIV infection in the follow-up between 29 weeks and 48 weeks. Of the 28 infants, 5 were excluded because of reported breastfeeding. Of the 23 HIV-infected infants remaining, 9 had PBMC specimens tested by ultrasensitive DNA PCR, which detected very low levels of HIV DNA in 6 of 9 infants up to 31 weeks earlier than with standard testing. Infants in the nevirapine arm had a median delay in HIV diagnosis of 22 weeks, infants whose mothers were receiving combination antiretroviral therapy had a median delay of 15 weeks, and infants in the control arm had a median delay of 9 weeks. Testing 6 weeks after reported cessation of breastfeeding (according to current WHO recommendations[5]) would not have captured 7 of the 9 infected infants. This suggests that repeat testing more than 6 weeks after cessation of breastfeeding may be needed.

Technau and colleagues reported data examining PCR testing at birth in South Africa, where national guidelines recommend targeted PCR testing at birth for all HIV-exposed neonates at risk for MTCT. In June 2014, universal testing of all HIV-exposed neonates was implemented in South Africa. A total of 14 infants had positive or indeterminate PCR test results, 6.7% of infants with targeted testing and 2.1% of infants with universal testing. Of the 8 neonates whose test results were indeterminate, 6 had positive results using other testing methods. Investigators found that targeted testing at birth would require testing of 52.3% of HIV-exposed infants but would fail to detect 35.7% of HIV infections compared with universal testing of all HIV-exposed infants at birth. These studies highlight the challenges of PMTCT and early infant diagnosis and reveal large gaps not only along the care cascade but also in the ability of current testing guidelines to detect HIV-infected infants.

Resistance to Antiretroviral Drugs

Transmitted Drug Resistance

Transmitted drug resistance (TDR) must be identified, as it can hamper the success of initial antiretroviral regimens. Rates and emerging trends in TDR among several populations were presented (Sessions P-L3 and P-L4). Investigators from Saskatchewan, Canada, described a large cluster of NNRTI-resistant transmitted virus among IDUs attending a large, regional infectious diseases clinic (Abstract 598). Molecular phylogenies were inferred based on anonymized bulk HIV-1 *pol* sequences from pretherapy genotyping of all

patients for whom testing was available, and clusters were mapped using superimposed resistance data. Of the 415 individuals in the analysis, a large transmission cluster of 81 individuals (19.5% of the clinic population) had pretherapy HIV with the G190A mutation in the reverse transcriptase gene, conferring NNRTI resistance. Compared with the overall clinic population, individuals with the G190A mutation in this cluster were more likely to be aboriginal (58/76 [76.3%]; RR, 1.5; $P < .01$), to have injection drug use as their primary risk behavior for acquisition of HIV (63/76 [82.9%]; RR, 1.5; $P < .01$), and to be coinfected with hepatitis C virus (64/76 [84.2%]; RR, 1.6; $P < .01$). The investigators urged consideration of these transmission dynamics in the context of social, cultural, and geographic factors, to mobilize effective public health and clinical resources.

Wang and colleagues presented estimates of TDR rates in New York from 2006 to 2013 based on recency of HIV infection (Abstract 599). Newly diagnosed cases of HIV infection were identified from the New York State HIV/AIDS surveillance registry and classified as recent, longstanding, or missing based on the results of a BED HIV-1 incidence enzyme immunoassay or evidence of longstanding HIV (ie, an AIDS diagnosis within 6 months of an HIV diagnosis). Cases were linked to genotype test results and TDR rates were generated. Of 13,015 newly diagnosed cases of HIV infection that had resistance test results available within 3 months of diagnosis, 2016 (15%) were classified as recent, 8703 (67%) were classified as longstanding, and 2296 (18%) were classified as missing. TDR rates among recently HIV-infected individuals rose from 17% in 2006 to 24% in 2013 (from 13% to 18% in cases with longstanding infection, and from 13% to 19% in all cases regardless of recency). The prevalence of TDR mutations was statistically significantly higher among recently infected individuals (19% vs 15%; prevalence ratio, 1.29; 95% CI, 1.16-1.43) across all subgroups (eg, sex, age, race and ethnicity, risk behavior, and geographic location). The investigators concluded that the recency of an HIV infection is an important variable and suggested that a growing number of HIV transmissions are attributable to treatment-experienced persons with poorly controlled HIV.

Rates of TDR in San Diego, California, were presented by Panichsillapakit and colleagues (Abstract 600). TDR rates were determined and case clustering analysis was performed by means of a retrospective analysis of pretherapy genotype testing from the San Diego Primary Infection Resource Consortium from 1996 through 2013. The overall prevalence of TDR was 16.2% (112/690; 95% CI, 13.6-19.2) and increased throughout the study period. TDR was chiefly observed for NNRTIs and increased over time (10.1% [70/690]; 95% CI, 8.0-12.7; $P < .001$, for trend). These findings highlight the value of surveillance for drug resistance and the importance of baseline resistance testing to guide treatment choices.

TDR rates in Mesoamerica were presented by Garcia-Morales and colleagues (Abstract 601). The analysis included HIV-infected, treatment-naive individuals enrolled between October 2010 and July 2014 from Mexico (n = 1476), Guatemala (n = 1180), Panama (n = 238), Nicaragua (n = 222), Honduras (n = 294), and Belize (n = 100). Plasma HIV *pol* sequences were obtained and HIV subtyping was performed. For most countries, TDR prevalence was intermediate (Mexico [7.7%], Guatemala [7.1%], Panama [12.2%], Nicaragua [14.9%], and Honduras [9.9%]) but was classified as high in Belize (19%). The investigators concluded that such findings indicate the need for ongoing local HIV molecular epidemiology and TDR surveillance studies. Garcia-Morales and colleagues also shared an individual country–specific analysis of antiretroviral drug resistance surveillance in Honduras after 10 years of widespread antiretroviral availability. There was an intermediate pretreatment drug resistance rate of 9.9%, of which resistance to NNRTIs accounted for 6.5% (Abstract 607).

Surveillance for TDR in rural KwaZulu-Natal, South Africa, was described by Manasa and colleagues (Abstract 603). The investigators reported on 3 rounds of annual population-based surveillance genotyping of treatment-naive patients from 2010, 2011, and 2012. Sequencing was performed on samples from 701 treatment-naive individuals: 67 (2010), 381 (2011), and 253 (2012). NNRTI resistance–associated mutations were the most dominant and were detected in 32 (5%) samples. The most common mutations were K103N (27, 3.8%), V106M (3, 0.4%), and G190A (2, 0.3%). Six (1%) of the participants had both NNRTI resistance– and nRTI resistance–associated mutations, K103N and M184V being the most common combination. There was no evidence of surveillance drug resistance mutations from the 2010 participants; however, baseline resistance was detected in samples from 2011 (5%) and 2012 (8%). The investigators concluded that ongoing surveillance of recently HIV-infected individuals is necessary to plan effective treatment coverage.

Olson and colleagues, on behalf of the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) collaboration, reported decreasing rates of TDR over time in its multisite cohort (Abstract 602). The cohort included HIV-infected, treatment-naive individuals with a recent seroconversion during the period from 1996 to 2012. There were 4183 subjects included in the analysis, which showed evidence of decreasing TDR to any drug class from 1996 to 2012 (OR, 0.918; 95% CI, 0.895, 0.942; $P < .001$, per year increase). The same trend was seen with nRTIs (OR, 0.881; 95% CI, 0.853, 0.911; $P < .001$), NNRTIs (OR, 0.965; 95% CI, 0.925, 1.008; $P = .11$) and PIs (OR, 0.915; 95% CI, 0.874, 0.958; $P < .001$). There was, however, evidence of an increased risk of TDR with acute HIV infection (OR, 1.18; 95% CI, 0.96, 1.46; $P = .12$). The investigators concluded that this last finding could suggest that TDR impacts seroconversion illness or that true TDR is underestimated if not tested for immediately after a seroconversion.

In addition to their common use for treatment-experienced individuals, InSTIs are increasingly used in initial antiretroviral regimens for treatment-naive, HIV-infected individuals. Chang and colleagues reported a troubling trend of increasing rates of TDR to InSTIs among treatment-naive patients with pretreatment genotype testing between June 2012 and December 2013 in Taiwan (Abstract 608). The overall prevalence of TDR to InSTIs was 6% (65/1087) and the most common mutations included Q148K and N155S, which were found in 41.5% (n = 27) and 29.2% (19), respectively, of InSTI-resistant strains. These findings contrast a 2011 analysis in Taiwan that did not show any HIV-1 strains with InSTI-related major mutations in a treatment-naive sample.

Use of Antiretroviral Drug Levels to Estimate True Levels of TDR

Chen and colleagues reported results from the HIV Prevention Trials Network (HPTN) 061 study and described HIV drug resistance patterns that were identified by antiretroviral drug screening (Abstract 116). HPTN 061, also known as the Brothers Study, enrolled HIV-infected and -uninfected black MSM who were at high risk for HIV infection in 6 cities in the United States (from 2009-2010). Most of the HIV-infected men in the study reported that they were unaware of their HIV serostatus or that they were aware of their serostatus but were not in care. HIV drug resistance was detected in 48 (28.4%) of 169 HIV-infected men who were not virally suppressed at time of study enrollment. A high-throughput assay was used to screen for the presence of antiretroviral drugs in samples from study participants. Samples were tested for the presence of 15 antiretroviral drugs using a qualitative assay based on high-resolution mass spectrometry.

An HIV Prevention Trials Network analysis found presence of antiretroviral drugs in 35.5% of newly diagnosed HIV-infected men, as well as statistically significant levels of drug-resistant virus.

Resistance results were contrasted with self-reported data on prior and current antiretroviral drug use for pre-exposure prophylaxis, postexposure prophylaxis, and antiretroviral treatment. Of 169 men with HIV infection, antiretroviral drugs were detected in 60 (35.5%), including 27 (56.3%) of the 48 men who had drug-resistant HIV. Unusual combinations of antiretroviral drugs were detected in the samples. Of 137 men who reported no prior or current antiretroviral drug use, 31 had drug-resistant HIV, suggesting a TDR rate as high as 22.6%; however, 14 of the men with drug-resistant HIV also had at least 1 antiretroviral drug detected.

In a second analysis, which excluded men with evidence of antiretroviral drug use, the estimated rate of TDR was 12.4%. Five (29%) of the 17 men with TDR had multiclass drug resistance. The investigators concluded that men were not disclosing their true antiretroviral drug use history and that concurrent antiretroviral drug testing along with resistance testing could provide a more accurate estimate of the rate of TDR.

Use of Untimed Antiretroviral Drug Levels to Predict Virologic Failure

Gonzalez-Serna and colleagues presented a retrospective analysis of untimed drug levels (UDLs) in patients with low-level viremia (LLV) in an effort to gain additional insight into patient outcomes after LLV (Abstract 117). Retrospective testing was performed on leftover samples from viral load or resistance testing. First documented LLV (defined as HIV-RNA level of 50 copies/mL-999 copies/mL) was analyzed for 2176 patient samples. Three hundred twenty-eight consenting patients also had drug level testing, genotypic resistance data, and follow-up clinical data available. Drug levels were characterized as therapeutic or suboptimal based on target trough concentrations from Department of Health and Human Services Guidelines.[6] The Stanford HIV Drug Resistance Database algorithm was applied to assess resistance. Of 328 patients, 78 (24%) had suboptimal drug levels during LLV and 63 (19%) had a genotypic susceptibility score (GSS) of less than 3. Suboptimal UDLs and a GSS of less than 3 independently increased the risk of having a future HIV RNA level of less than 1000 copies/mL. Within 1 year, 56 of 78 (72%) patients with suboptimal UDLs experienced virologic failure, compared with 45 of 63 (71%) patients who had GSSs of less than 3, and 103 of 206 (50%) patients who had optimal GSSs and UDLs. Of patients with suboptimal UDLs, 43 of 78 (55%) had undetectable levels of PIs or NNRTIs, with most (81%) patients experiencing virologic failure by 1 year. Only 18 patients had both suboptimal UDLs and a GSS of less than 3. The investigators concluded that suboptimal UDLs are one of the strongest predictors of time to virologic failure and pointed out that subtherapeutic drug levels were associated with the presence of resistance and with virologic failure. A single plasma UDL may enhance prediction of subsequent virologic failure, as low drug levels are more common and better predictors than resistance data during LLV. When used together, UDLs and GSSs can explain a higher proportion of treatment failures than either measure used alone. These results could justify the potential investigation of UDLs in the prospective management of LLV.

Untimed antiretroviral drug levels are a strong predictor of subsequent virologic failure.

Drug Resistance and Transmission Networks

Seeking to explore how drug resistance–associated mutations affect transmission, Wertheim and colleagues presented analysis of fitness effects of drug-resistant strains across a US HIV-1 transmission network (Abstract 120). The investigators analyzed 66,235 HIV-1 *pol* sequences reported to the US National HIV Surveillance System of persons diagnosed with

HIV infection through 2012. Nearly half of the samples were collected from antiretroviral treatment–naive persons within 3 months of diagnosis. Sequences were aligned using a reference sequence, resistance mutation–associated codons were removed, and a transmission network was constructed so that clustering could be determined by the presence of resistance-associated mutations. Of 30,200 antiretroviral treatment–naive persons, 12,539 (42%) clustered. Clustering was not associated with PIs and NNRTIs; however, nRTI resistance–associated mutations were associated with reduced clustering compared with strains without resistance-associated mutations ($P < .0001$). This finding persisted even after adjustment for age, race and ethnicity, transmission category, geographic region, and diagnosis year. The M184V mutation, which has known adverse fitness consequences, was associated with a lower prevalence of clustering (18%) and was present in only 8% of persons with an nRTI resistance–associated mutation. Many nRTI resistance–associated mutations were found to contribute to the overall effect; M41L, T69N, D67N, and M184V were the greatest contributors.

The study also revealed that specific PI resistance–associated mutations could increase or decrease clustering, as illustrated by the finding that L90M strains cluster statistically significantly more than those without this mutation. Further, after excluding L90M, other PI resistance–associated mutations were associated with statistically significantly reduced clustering. Similarly, the K103N/S strains clustered more but, after excluding these, other NNRTI resistance–associated mutations substantially reduced clustering. The investigators pointed out that mutations (M184V and K65R) that affect approved preexposure prophylaxis regimens are infrequently transmitted, which is reassuring. However, except for the nRTI mutations, resistance-associated mutations did not reduce interhost transmission. The investigators also pointed out the propensity for drug-resistant HIV-1 to spread depending on the mutation.

Evaluation of Second-Line Treatment

Boender and colleagues presented data from the PASER-M (Pan-African Studies to Evaluate Resistance Monitoring) study (Abstract 118). The study investigated the impact of pretreatment drug resistance (PDR) on 2- and 3-year antiretroviral therapy outcomes and of switching to a second-line regimen in the first 3 years of antiretroviral therapy. The PASER-M study followed HIV-infected individuals initiating antiretroviral therapy for 2 years (13 sites) or 3 years (5 sites) in 6 African countries. Viral load and *pol* genotypic testing (if HIV RNA level > 1000 copies/mL) was performed at initiation of antiretroviral therapy and then annually. PDR was defined as a decreased susceptibility to at least 1 prescribed drug, using the Stanford HIV Drug Resistance Database algorithm and the International Antiviral Society–USA (IAS–USA) list of drug resistance mutations in HIV-1.[7] The effect of PDR on switching to a second-line antiretroviral regimen with acquired drug resistance, virologic failure (HIV RNA level > 400 copies/mL), and acquired drug resistance during the first 3 years of antiretroviral therapy were assessed. Baseline genotype testing was available for 2570 participants at initiation of antiretroviral therapy, of which 5% (n = 139) had demonstrated PDR. After 3 years, 112 (4.3%) participants had switched to a second-line antiretroviral regimen; 78 (69.6%) of these had genotype testing results available and one-third (n = 26) had switched regimens unnecessarily. PDR increased the risk of a regimen switch with drug resistance (subhazard ratio, 7.8; 95% CI, 3.9-15.6) during 3 years of initial antiretroviral therapy; risk of virologic failure after 2 years (OR, 2.9; 95% CI, 1.4-5.8) and 3 years (OR, 2.8; 95% CI, 1.1-7.2) of initial antiretroviral therapy; and risk of acquired drug resistance after 2 years (OR, 2.5; 95% CI, 1.2-5.4) and 3 years (OR, 5.0; 95% CI, 1.8-14.3) of initial antiretroviral therapy. Interestingly, PDR was not associated with mortality or new AIDS events in this cohort. The investigators concluded that viral load monitoring can enable timely detection of treatment failure and avoid unnecessary regimen switches. Such findings have important implications for allocation of antiretroviral resources.

Paton and colleagues presented data on the impact of drug resistance on second-line antiretroviral treatment in Africa from the EARNEST trial (Abstract 119). The study included 1277 patients aged 12 years or older who met treatment failure criteria after more than 12 months of NNRTI-based, initial antiretroviral therapy in rollout programs at 14 sites in 5 African countries. Patients who experienced treatment failure with an NNRTI- or nRTI-based regimen were randomly assigned to receive 1 of 3 regimens: a PI plus 2 or 3 nRTIs, a PI plus an InSTI (raltegravir), or PI monotherapy. Resistance testing was performed on batched stored samples. Patients demonstrated advanced treatment failure with a predicted susceptibility to nRTIs of 0 in 230 (59%) patients, to 1 active nRTI in 128 (33%) patients, and to 2 or more active nRTIs in 33 (8%) patients. Of individuals randomly assigned to receive a PI plus 2 or 3 nRTIs, the rate of suppression was comparable to that of individuals receiving a PI plus raltegravir (76% vs 72%, respectively; $P = .28$) and far exceeded the rate of suppression of individuals receiving PI monotherapy (76% vs 44%, respectively; $P < .001$).

Even without predicted activity owing to resistance, nRTIs contributed to the efficacy of second-line regimen with a PI and 2 or 3 nRTIs and clearly added activity compared with PI monotherapy that was equivalent to adding a drug from a new class. The investigators suggested that this could be attributable to a fitness effect, that algorithmic nRTI drug selection and attention to adherence are likely to achieve optimal outcomes in standardized second-line antiretroviral therapy with a PI and 2 to 3 nRTIs in resource-limited settings, and that using resistance testing to select nRTIs would add little value.

InSTIs and Resistance

Several presentations highlighted important concepts in HIV resistance to

InSTIs. Huang and colleagues reported on the combined effects of primary mutations that enable HIV to escape dolutegravir drug pressure (Abstract 121). In the study, a panel of site-directed mutations of HIV-1 integrase, including Y143R, Q148H, and N155H, in combinations or with secondary mutations was used. InSTI susceptibility and replication capacity were then measured. Although single mutations at positions 143, 148, and 155 did not confer reductions in dolutegravir susceptibility, the combined Y143R plus N155H and Q148H plus N155H pathways did result in modest reductions in dolutegravir susceptibility (fold change of 3.1 and 4.2, respectively). Viruses harboring mutations belonging to the 148 plus 155 escape pathway were less susceptible to dolutegravir, with similar or greater replication capacity than the 143 plus 148 or 143 plus 155 pathways.

HIV-1 variants with combinations of mutations at integrase positions 143, 148, and 155 exhibit reduced susceptibility to dolutegravir, and the combined 148 plus 155 mutation pathway results in the least susceptibility.

The addition of G140S, a Q148 pathway mutation, to the double mutants Y143R plus Q148H and Q148H plus N155H further reduced dolutegravir susceptibility (fold change of 6.2 and 35.0, respectively) and fully restored the replication capacity of the viruses with Y143R plus Q148H mutations and those with Q148H plus N155H mutations (replication capacity, 17% and 3%, respectively). The investigators concluded that in the face of dolutegravir pressure, HIV-1 variants with combinations of mutations at positions 143, 148, and 155 exhibit reduced susceptibility to dolutegravir, and the combined 148 plus 155 mutation pathway results in the least susceptibility. Viruses with the 148 plus 155 combination of mutations likely possess a replication advantage over other mutation pathways.

Vavro and colleagues compared baseline integrase genotypic and phenotypic correlates to day 8 and long-term treatment responses using pooled data from the VIKING-3 and VIKING-4 studies (Abstract 609). These studies examined the use of dolutegravir in HIV-infected adults with multiclass antiretroviral drug resistance, including resistance to InSTIs. Three derived baseline integrase genotypic groups were identified: having no Q148 mutations, presence of the Q148 mutation plus 1 resistance-associated mutation, or presence of the Q148 mutation plus 2 or more resistance-associated mutations. The investigators reported that these groups were good predictors of dolutegravir response through week 48 and suggested that this analysis provides guidance for the clinical use of dolutegravir in patients with InSTI-resistant virus.

Theys and colleagues reported discordant clinical predictions of dolutegravir effectiveness and raised concerns about the complexity of mutational patterns that could lead to uncertainty in individual patient management (Abstract 610). Investigators analyzed 215 HIV-1 integrase sequences of patients whose treatment with raltegravir was failing and identified InSTI resistance–associated mutations, defined based on the IAS–USA 2013 list of drug resistance mutations in HIV-1.[8] Mutations were then interpreted via 5 resistance interpretation systems: the resistance interpretation systems ANRS v23, HIVdb v7.0, and Rega v9.1.0, and using US FDA and European Medicines Agency package inserts for raltegravir, elvitegravir, and dolutegravir. There was substantial disagreement in predicting resistance among the 5 scoring systems in 34.7% of patients, raising concern regarding interpretation and clinical management for individual patients.

Doyle and colleagues from the CORONET study group examined the influence of HIV-1 subtype on the pathways of genotypic resistance to InSTIs (Abstract 594). Integrase sequences produced using Sanger sequencing at 9 clinical centers were analyzed centrally to identify major InSTI resistance–associated mutations, as defined by the Stanford HIV Drug Resistance Database algorithm. There were 255 sequences from raltegravir-experienced patients (82% with HIV subtype B) and 533 from raltegravir-naive patients (75% with HIV subtype B). Non-B subtypes included 11 different variants. Subtype B variants had a higher propensity to develop the G140 and Q148 pathway than predominantly non-B subtypes that was attributed to a different codon usage at the G140 position. The investigators noted that these findings have implications for use of dolutegravir in people who have taken other InSTIs.

Hassounah and colleagues described a simian immunodeficiency virus (SIV) model of HIV drug resistance against InSTIs (Abstract 591). Drawing on previous work showing that SIV_{mac239} is susceptible to raltegravir, elvitegravir, and dolutegravir,[9] the investigators sought to assess the similarities in resistance pathways between SIV and HIV under InSTI pressure. Selections in tissue culture were performed in the PBMCs of rhesus macaques infected with SIV_{mac239} in the presence of raltegravir, elvitegravir, and dolutegravir. Viral RNA was extracted from cell culture supernatants and sequenced for any changes in the integrase coding region. The integrase gene was cloned into a bacterial expression vector and resistance mutations were introduced by site-directed mutagenesis. Purified recombinant SIV_{mac239} wild-type G118R, Y143R, Q148R, N155H, or R263K integrase enzymes were obtained and strand transfer activities were assessed. Several known dolutegravir-associated HIV mutations decreased the activity of dolutegravir on SIV. The investigators suggested that these data support the use of this nonhuman primate model to study HIV pathogenesis, therapy, and transmission. SIV_{mac230} viruses treated with dolutegravir led to the emergence of the R263K mutation, similar to the unique pattern of dolutegravir resistance that has been seen in HIV-infected study subjects. This analysis confirms that the same mutations associated with drug resistance in HIV exhibit similar profiles in SIV.

Next-Generation Sequencing

A Themed Discussion on next-generation sequencing (Session TD-B) included several techniques for identifying drug resistance. Boltz and colleagues analyzed resistance haplotypes using primer IDs and next-generation sequencing of HIV RNA (Abstract 593). Aiming to address the challenges of PCR bias and recombination, Boltz described a new method for library construction that produces large numbers of tagged consensus sequences, allows for increased sensitivity of haplotype determination, and reveals the source of recombination. The analysis compared the PCR primer method with a new ligation method to sequence DNA. The ligation method entails use of 22-mer uracil-containing primers followed by digestion, cleavage, and ligation to linkers containing sequences. Utilizing paired-end MiSeq Illumina technology, DNA was sequenced and consensus sequences were derived from a supermajority (≥80% consensus) for each unique ID. Consensus sequences were analyzed for PCR bias, errors, recombination, and sensitivity in detecting haplotypes. Using synthesized complementary DNA (cDNA) from mixtures of cloned wild-type and mutant HIV-1 *pol* transcript RNA, the methods were compared. The newer ligation method showed an even distribution of amplified templates with statistically significantly less PCR bias than the primer method. The PCR recombination rate for the ligation method was 0.01% compared with 0.16% for the primer method and was able to detect drug resistance mutations down to 0.001% and was only 0.01% with the primer method. The sensitivity of haplotype detection was also better with the ligation method; for samples containing 10% or 1% mutants, the primer method never detected linkage of all 14 mutations, whereas the ligation method did detect all 14 mutations.

Although HIV-1 genotyping is an important tool for clinical and epidemiologic studies, standard methods are associated with high levels of genetic variation, recombination, and mutations that pose difficulty in successful PCR amplification of HIV-1 genomes. Additionally, emerging new subtypes may not be detected with standard PCR primers. With these challenges in mind, Ragupathy and colleagues presented a novel PCR-free multiplex method for characterization of full-length HIV-1 genomes (approximately 9.7 kb) using the next-generation RNA sequencing approach (Abstract 258). This approach enabled accurate reconstruction of whole-genome HIV-1 haplotypes, including flanking long terminal repeats. Analysis of full HIV-1 genome sequences using similarity plotting correctly identified 15 pure subtypes, 1 group O virus, and recombination patterns of 8 circulating recombinant forms and 3 unique recombinant forms. All HIV subtypes identified were comparable with those seen using Sanger sequencing. The multiplex RNA sequencing approach revealed NNRTI, InSTI, and PI drug-specific minor variants, drug-resistance mutations, and tropism status. The investigators concluded that HIV-1 genotyping using RNA sequencing is feasible, accurate, and offers the advantage of not requiring prior knowledge of the genome sequence.

Berg and colleagues also presented a next-generation sequencing strategy for viral surveillance, the HIV-SMART approach (Abstract 257). This gene-specific approach does not require apriori knowledge of subtype or group. It is a universal approach that requires 2 days to construct libraries and entails specific amplification of HIV followed by deep sequencing of the virus isolates and samples. Reverse transcription primers, designed in conserved regions of HIV and spaced at 1.5 kb to 2 kb intervals, fuse viral sequences to a common adaptor (SMART) sequence. This same adaptor sequence is added to the 3′ end of the cDNA to permit PCR amplification of libraries, which are then tagmented for multiplexing and sequencing. HIV sequences are extracted and assembled using software and classified by phylogenetic analysis. This technique was applied to 47 virus isolates, and in a single run that multiplexed 23 libraries there was 100% genome coverage and the technique was able to capture extensive diversity.

Financial affiliations in the past 12 months: Drs Olender, Taylor, and Wong have no relevant financial affiliations to disclose. Dr Wilkin has served as a consultant to GlaxoSmithKline/ViiV Healthcare, has received research support awarded to his institution from Gilead Sciences, Inc, Bristol-Myers Squibb, and GlaxoSmithKline/ViiV Healthcare, and has received travel support from GlaxoSmithKline/ViiV Healthcare. His spouse is an employee of Johnson and Johnson.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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Release date: Monday, February 16, 2015.
CME Credit Available: **1.50** *AMA PRA Category 1 Credits*™
Level: **Advanced**

Depression is one of the most common causes of years of life spent living with disability globally, and is considerably more prevalent among people with HIV infection than in the non–HIV-infected population. Moderate to severe depression among people with HIV infection is associated with increased substance use, poor functioning, poor quality of life, and suicidal behavior, as well as many negative HIV-related outcomes, including increased risk behaviors that could lead to HIV transmission, failure to access HIV care and treatment, failure to adhere to HIV care and treatment, more-rapid progression of HIV illness, and increased morbidity and mortality. There is evidence that being in treatment for depression is associated with improvements in these HIV-related outcomes. In addition, treating depression successfully reduces suffering and disability.

Prevention of Cardiovascular Disease in the HIV-Infected Individual
Michelle Zikusoka, MD, MHS; Wendy S. Post, MD, MS; Todd T. Brown, MD, PhD
CME Credit Available: **1.50** *AMA PRA Category 1 Credits*™
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Given the prevalence of traditional risk factors, the aging of HIV-infected individuals, and the potential cardiovascular consequences of HIV disease and antiretroviral therapy, more attention should be focused on efforts to decrease the morbidity and mortality associated with atherosclerotic cardiovascular disease (ASCVD) and HIV infection. The key to success in CVD prevention is education of the clinician and the patient.

Drug Interactions With Hepatitis C Virus Direct-Acting Antivirals
John J. Faragon, PharmD
CME Credit Available: **1.50** *AMA PRA Category 1 Credits*™
Level: **Advanced**

The availability of newer direct-acting antiviral (DAA) drugs in the management of hepatitis C virus (HCV) infection has led to dramatic increases in sustained virologic response rates among patients receiving treatment with these drugs. Although these newer HCV DAAs have improved response rates, their use may result in unexpected drug interactions with primary care medications and many HIV antiretroviral drugs. However, the risk of drug interactions with the new DAAs is likely to be lower than with the earlier DAAs boceprevir and telaprevir.

Osteomalacia and Osteoporosis in the HIV-infected Patient
Michael T. Yin, MD; Emily M. Stein, MD, MS
CME Credit Available: **1.50** *AMA PRA Category 1 Credits*™
Level: **Advanced**

HIV-infected individuals, especially those on antiretroviral therapy, have lower bone mineral density (BMD) as measured by dual-energy x-ray absorptiometry (DXA) than HIV-uninfected individuals of similar age. However, low BMD can be the result of either osteomalacia, a disease characterized by impaired mineralization of bone matrix, or osteoporosis, a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to skeletal fragility. Differentiating between the 2 processes is important because they have different treatments. Treatment of HIV-infected individuals with low BMD is especially challenging because of antiretroviral effects on vitamin D and bone metabolism.

Novel HIV-1 Resistance and Tropism Testing
Jonathan Li, MD
CME Credit Available: **1.25** *AMA PRA Category 1 Credits*™
Level: **Advanced**

The remarkable diversity of HIV stems from a high replication rate and the error-prone reverse transcriptase enzyme used to translate HIV RNA into DNA. Up to 5 mutations may arise with each new HIV virus produced; more than a billion new virions may be produced daily in a chronically infected patient. This diversity and rapid evolution allow HIV drug resistance to emerge in patients who are on antiretroviral therapy that is not adequately suppressive or who are not fully adherent to their antiretroviral regimen. When available, HIV drug–resistance testing should be used to guide the selection of an optimal antiretroviral regimen.

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Review

Neurologic Complications of HIV Infection

Serena S. Spudich, MD; Beau M. Ances, MD, PhD

More than 30 years into the HIV epidemic, research efforts are focusing on better understanding how the central nervous system (CNS) is adversely affected by HIV and on improving the quality of life of HIV-infected individuals. At the 2015 Conference on Retroviruses and Opportunistic Infections, neurologic presentations concentrated on characterization of potential CNS reservoirs of HIV, the pathogenesis of HIV-associated neurocognitive disorders (HAND), diagnosis of cognitive dysfunction caused by HIV, neuroimaging biomarkers of HAND, and treatment of modifiable risk factors of HAND. Studies presented also highlighted research on CNS disorders in international, resource-limited settings, setting the stage for a growing collection of collaborative studies that will directly impact the largest concentrations of people living with HIV worldwide.

Keywords: CROI 2015, HIV, central nervous system, HIV-associated neurocognitive disorder, neuroimaging, neuropathogenesis, cerebrospinal fluid

A potentially deleterious impact of HIV on the nervous system in otherwise stable-appearing individuals has garnered growing concern. HIV may establish infection and immune activation in the nervous system early during infection, and recognition of these early events has implications for potential persistent sites of HIV infection and for early and long-term neuropathogenesis.[1] Although the incidence of more advanced forms of HIV-associated neurocognitive disorders (HAND) has declined with the use of potent antiretroviral therapy, milder forms of HAND remain relatively common.[2,3] Further, HIV infection and associated inflammation may persist in the central nervous system (CNS) in some individuals during antiretroviral treatment. Studies presented at the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 22 to 26, aimed to unravel the biologic underpinnings of HIV effects in the CNS and to develop therapeutic strategies to address ongoing abnormalities related to these in HIV-infected individuals.

Central Nervous System HIV Persistence and Latency: Evidence, Measurements, and Mechanisms

Several studies at CROI 2015 focused on characterizing and interrogating HIV in CNS tissues in order to shed light on the possibility and nature of a CNS reservoir. Gianella and colleagues (Abstract 58) examined genetic attributes of paired cerebrospinal fluid (CSF) and plasma samples from 14 HIV-infected men after interruption of antiretroviral therapy. This study investigated the patterns of genetic compartmentalization between CSF and blood that might reveal the source of HIV rebound after interruption of therapy.

Study participants had been on antiretroviral therapy for more than 4 years, although whether they were all virally suppressed during this period was unknown. Next-generation sequencing using the 454 platform was employed to examine viral sequences (including *env, gag,* and *pol*) in CSF and blood after treatment interruption. HIV rebounded first in blood and then in CSF. Rebounding HIV was occasionally equilibrated, meaning that the HIV found in CSF was identical to that found in blood. However, in many participants (10/14), HIV rebounding in CSF was distinct from that in blood in at least 1 gene, most typically *env*. Although this was a retrospective study with variable timing of sampling after treatment interruption, the 4 participants who had samples taken in the first weeks after treatment interruption all had compartmentalization of rebounding HIV. In 11 participants, serial sampling after treatment interruption provided a window to study if compartmentalization in the CSF was sustained; in a majority of these, the compartmentalization persisted. These studies suggest a source of HIV in CNS independent from blood after treatment interruption. Methods such as these, including sampling after treatment interruption and the use of next-generation sequencing, are likely to be powerful tools for dissecting compartmentalized sources of HIV and their characteristics in future studies.

A key challenge to assessing HIV persistence in the CNS compartment is the need to quantify HIV in the CNS in HIV-infected participants, using current tissue sampling strategies. To date, most studies have relied on measuring cell-free HIV RNA from CSF supernatant to provide an assessment of HIV burden in the CNS. Hellmuth and colleagues (Abstract 438) compared levels of HIV RNA in CSF and blood, using standard viral load assays (lower limit of detection of 50 copies/mL in blood and 100 copies/mL in CSF) in HIV-infected individuals during acute and chronic infection, with a

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higher ratio of plasma to CSF HIV RNA observed during early than in chronic infection. Further, 10 of 42 individuals assessed during acute HIV infection had levels of HIV RNA in CSF that were below the limit of detection and were associated with lower levels of biomarkers of immune activation in plasma and CSF. This study raises the question of whether initiation of antiretroviral therapy during acute HIV infection may reduce the burden of persistent CNS infection or prevent dissemination to the CNS, given the lack of detectable HIV RNA in the CNS in 24% of individuals during this period.

Two studies that also focused on HIV RNA recovered from the CSF suggested a relationship between the extent of local HIV replication within the CNS compartment and neuropathogenesis. Joseph and colleagues (Abstract 440) examined the genetic attributes and phylogenetic relationships of HIV in paired CSF and blood samples from 40 individuals with neuropsychologic testing–based diagnoses of distinct categories of HAND. In some individuals, partial *env* genes were amplified using deep sequencing with a primer ID, and in others, full-length *env* was amplified by single genome amplification. The investigators found increasing proportions of CNS compartmentalization with increasing severity of HAND: 29% in neurologically normal participants, 40% in participants with asymptomatic neurocognitive impairment (ANI) or mild neurocognitive disorder (MND), and 70% in participants with HIV-associated dementia (HAD). Using an Affinofile assay to determine putative macrophage infectivity of HIV based on entry into cells with varying surface levels of CD4 receptors, the investigators determined that 71% of compartmentalized viruses derived from CSF appeared to be adapted to replication within macrophages.

Robertson and colleagues (Abstract 439) also used CSF HIV RNA as a measure of CNS HIV infection, using the HIV RNA level as a proxy for extent of CNS infection in a group of approximately 30 individuals evaluated with CSF collection and neuropsychologic testing before and after antiretroviral therapy (regimens were determined separately from the study). A lumbar puncture and neuropsychologic testing (11 tests, normed and averaged to yield a total *z* score) were obtained prior to treatment, with repeat testing at intervals following antiretroviral therapy initiation: lumbar puncture at 2 weeks to 4 weeks and neuropsychologic testing at 24 weeks. The median CSF HIV RNA level of 3.14 $\log_{10}$ copies/mL at baseline was reduced to 1.60 $\log_{10}$ copies/mL after 2 weeks of antiretroviral therapy, and the baseline summarized total *z* score of -0.91 had improved to -0.71 at the time of follow-up.

Analysis demonstrated relationships between the degree and rapidity of HIV RNA reduction in the first 2 weeks of antiretroviral treatment and the extent of improvement in neurocognitive functioning during the first 6 months after initiating therapy, suggesting that early attenuation of HIV RNA replication in the CNS may lead to enhanced neurocognitive responses to treatment. Alternatively, these data could suggest that individuals with a slower or less pronounced reduction in HIV RNA initially have a more severe local CNS HIV infection, which is associated with poorer long-term reversal in response to standard antiretroviral treatment.

CSF HIV RNA is typically reduced to below levels of standard detection in individuals taking antiretroviral treatment. Attention has recently turned to whether HIV nucleic acid detected in cells recovered from the CSF may be a useful measure of HIV persistence in the CNS. de Oliviera and colleagues (Abstract 435) measured *pol* using a droplet digital polymerase chain reaction (ddPCR) assay, to quantitate HIV DNA in peripheral blood mononuclear cells (PBMCs) and CSF cell pellets obtained from 29 HIV-infected individuals. Twenty of these individuals were taking antiretroviral therapy for a median of 2.3 years and were virologically suppressed, with HIV RNA levels in blood and CSF of less than 50 copies/mL. ddPCR yielded detectable HIV DNA in 66% of CSF cell pellet samples from the entire group, and in 50% of CSF samples from individuals with undetectable HIV RNA in both compartments. Although a higher proportion of PBMC samples had detectable HIV DNA (100% of the overall group), median levels of HIV DNA were similar or higher in CSF (3.4 $\log_{10}$ copies/million cells) than in PBMCs (2.2 $\log_{10}$ copies/million cells) in individuals taking suppressive antiretroviral therapy.

In the group overall, higher levels of HIV DNA in CSF correlated with higher levels measured in PBMCs and with higher levels of HIV RNA in CSF. More than 4.0 $\log_{10}$ copies/million CSF cells were detected in some individuals with undetectable HIV RNA levels, indicating persistence of HIV in CNS cells during suppressive antiretroviral therapy. These data provide rationale for future studies focused on characterization of HIV detected in CNS cells during suppressive antiretroviral therapy, to potentially yield further insight into the sites and mechanisms of HIV persistence.

In further studies examining HIV persistence and latency in the CNS, Gelman and colleagues (Abstract 61) studied brain specimens from 40 individuals who died with HIV infection and from 20 HIV-uninfected individuals, using autopsy materials obtained through the National NeuroAIDS Tissue Consortium. In order to determine tissue biomarkers and mechanisms associated with HIV DNA in the brain, the investigators examined the relationship between macrophage markers associated with and levels of HIV DNA relative to HIV RNA measured in the dorsal prefrontal cortex, an area often affected by HIV. Although a number of the markers considered standard indicators of macrophages and microglia (including CD16, CD14, and CD163) did not differentiate between those with proportionally higher HIV DNA, other markers (including interferon regulatory factor 4 [IRF-4]; C-type lectin domain family 4, member A [CLEC4A, also termed DCIR]; and interleukin 10 [IL-10]) were higher in this group.

These results are of interest because IRF-4 is a transcription factor that could

potentially mediate the relationship between integrated HIV DNA and expressed HIV RNA. IRF-4 is also regulated by a polycomb repressive complex such that the methyltransferase enhancer of zeste homolog 2 (EZH2) suppresses the expression of IRF-4; thus, if important as a regulator, EZH2 might lower HIV DNA relative to HIV RNA. Dual staining of macrophages in the leptomeninges of the brains of these individuals showed that the presence of EZH2 was associated with increased expression of HIV RNA, suggesting that this pathway might be related to control of viral expression versus maintenance of HIV DNA in a latent state. Although these analyses were performed in autopsy studies of HIV-infected individuals who were not necessarily taking suppressive antiretroviral therapy at the time of death, these results reveal potentially important mechanisms of endogenous regulation of HIV DNA transcription in the human brain.

Another study presented at CROI 2015 that was focused on brain autopsy tissue explored an issue of relevance to control of HIV replication in the setting of antiretroviral treatment. In order to assess how antiretroviral drugs may access brain tissue and potentially impact levels of HIV infection in this compartment, Bumpus and colleagues (Abstract 436) examined samples from 3 brain regions (globus pallidus, cortical grey matter, and white matter) obtained from autopsy studies from the California NeuroAIDS Tissue Consortium. Concentrations of atazanavir, efavirenz, emtricitabine, and lamivudine in the brain were similar to those reported in CSF. However, tenofovir, which is considered to have potentially poor CNS efficacy owing to low measured concentrations in the CSF, had concentrations that were notably higher in all brain regions than in the CSF. Lopinavir concentrations were also higher in the brain in the frontal white matter than in the CSF. This study reveals that although concentrations of certain drugs reaching the brain can be extrapolated from levels found in the CSF, in some cases CSF may underestimate concentrations in the brain tissue compartment. Further studies focused on measuring not only whole drug concentrations but also concentrations of phosphorylated nucleotide analogue reverse transcriptase inhibitor anabolites may further enhance precision of antiviral activity estimates in the CNS.

New approaches for assessing HIV persistence or replication in the CNS include comparison of HIV variants in compartments after treatment interruption, ddPCR measurement of CSF fluid cell-associated DNA, and next-generation sequencing to genetically characterize HIV in the CNS compartment.

Gama and colleagues (Abstract 416) examined issues of latency and explored strategies for reducing persistent lentiviral integration in the CNS by employing latency-reactivating agents in neurologic studies of rhesus macaques. Using an accelerated macaque model of neuroAIDS created by dual infection with 2 neurovirulent strains of simian immunodeficiency virus (SIV; SIVDeltaB670 and SIV/17-Fr), these investigators treated 3 animals with antiretroviral therapy such that they were virally suppressed to less than 100 copies/mL of SIV RNA in plasma for 500 days. They then exposed 2 of the animals to the protein kinase C activator ingenol-3-hexanoate (Ing-B, a putative latency-reactivating agent) for 40 days, allowed a 2-week washout period, and then exposed these animals to Ing-B plus vorinostat (total 6 mg/kg) for 15 days. One control animal received antiretroviral therapy alone without Ing-B or vorinostat.

CSF assessments were obtained throughout the study, and the animals were euthanized for examination of brain tissue after the interventions, with measurement of CSF and plasma SIV RNA by quantitative PCR (qPCR) and ddPCR, measurement of SIV DNA in brain by qPCR, and measurements of SIV RNA in brain by in situ hybridization. One animal treated with latency-reactivating agents had a marked rise in plasma and CSF SIV RNA after treatment with the combination of agents, with a 10-fold higher SIV RNA level in CSF than in plasma and development of SIV encephalitis despite continued treatment with antiretroviral therapy. This animal also had increases in levels of CSF neopterin (a marker of macrophage activation), chemokine (CC motif) ligand 2 (CCL2; a marker of monocyte chemoattraction), and neurofilament light chain (NFL; a marker of neuronal injury) after intervention. Moreover, SIV RNA was elevated in the occipital cortex in this animal at sacrifice, and a quantitative outgrowth assay to determine absolute number of infected resting CD4+ T cells harvested from PBMCs revealed that in both treated animals, there was a decline in infected resting CD4+ T cells after use of latency-reactivating agents. Although a small study in a simian model developed for study of encephalitis and thus of accelerated disease with uncertain generalizability to human CNS HIV infection, this is the first study to demonstrate the activity of latency-reactivating agents in the CNS and the potential deleterious effects of this strategy in the CNS compartment.

Mechanisms of Neuropathogenesis in HIV: Immune Activation, Mitochondrial Dysfunction, and Toxicity

That activation of the host immune system resulting in inflammatory-mediated damage in the CNS is a key substrate of HIV-related neurologic injuries is well established. However, the details of the processes and pathways involved in this immune response are still not well understood, and must be determined in order to develop effective therapies for injuries to the nervous system in individuals infected with HIV. As a parallel process to cellular infiltration and soluble inflammatory mediator activation in the CNS, perturbation of blood-CNS barriers may serve an important role in neuropathogenesis of HIV, by allowing increased influx of infected and activated cells and of toxic soluble products into the CNS.

Two oral presentations at CROI 2015 examined the status of the blood-CNS barrier in HIV-infected individuals, using a measure of albumin concentration in CSF compared with blood (CSF-to–plasma albumin ratio). Anesten and colleagues (Abstract 59) measured CSF-to–plasma albumin ratio in 657 HIV-infected individuals categorized by HIV treatment status, and by clinically based diagnosis of HAD versus lack of neurologic symptoms (neuroasymptomatic) in individuals not taking antiretroviral therapy. Neuroasymptomatic individuals were further divided based on CD4+ cell count range. When albumin ratio in each HIV-infected group was compared with that in a group of 53 HIV-uninfected controls who had CSF samples collected for research purposes, statistically significant elevations were only found in the group with HAD. No differences were noted across the CD4+ cell count spectrum between HIV-uninfected controls and HIV-infected individuals whether they were or were not taking suppressive antiretroviral therapy.

When compared with age-determined published cutoffs of upper limit of normal albumin ratio, abnormally elevated levels were detected in 16% of participants in the neuroasymptomatic group that was not taking antiretroviral therapy and in 68% of individuals with HAD. Despite albumin ratios within the normal range in most individuals in the study, NFL (as noted above, a marker of active neurologic injury) level correlated with albumin ratio in the HIV-infected groups, and in the antiretroviral treated, virally suppressed group. In a multivariate model, albumin ratio was a predictor of NFL level independent of age. These results indicate that blood-CNS barrier disruption is associated with neurologic injury in HIV infection, and suggest that this process is a late-stage complication of HIV that is specifically related to severe encephalitis and dementia.

In a longitudinal study of primary HIV infection—defined as within the first year of HIV acquisition—Rahimy and colleagues (Abstract 62) examined the CSF-to–plasma albumin ratio. Albumin ratio measured at baseline (a median 3 months postinfection) in 108 individuals with primary HIV infection was elevated compared with an age-matched group of HIV-uninfected individuals. Over a median 1 and one-half years of longitudinal follow-up prior to initiation of antiretroviral therapy, CSF-to–plasma albumin ratios did not change, suggesting a lack of resolution of blood-CNS barrier disruption during this early period. Moreover, in a smaller cohort (n = 57), the CSF-to–plasma albumin ratio did not statistically change 1 year after initiating antiretroviral therapy. The CSF-to–plasma albumin ratio correlated with CSF NFL level in individuals with primary HIV infection at baseline and in longitudinal follow-up, and inversely correlated with N-acetylaspartate–to-creatine ratio, a neuroimaging measure of neuronal integrity in the parietal grey matter.

Differing results between this study and those noted in Abstract 59 may relate to a lack of age matching in HIV-uninfected comparison participants in the larger study, or to a distinct and dynamic pattern of blood-CNS perturbation that is specific to early HIV infection. In this same cohort of primary HIV infection, Wright and colleagues (Abstract 60) demonstrated that a higher CSF-to–plasma albumin ratio correlated with reduced putaminal volume. These data underscore the potential pathogenic significance of blood-CNS barrier perturbation in CNS and emphasize that processes in HIV infection associated with neurologic damage and neurocognitive impairment are initiated during the early stages of infection.

Edén and colleagues (Abstract 474) focused on the concept that progressive neurologic injury in the CNS in individuals taking suppressive antiretroviral therapy may relate to underlying mechanisms of persistent immune activation. They explored measures of intrathecal macrophage activation (CSF neopterin) and neuronal injury (CSF NFL) in 100 individuals taking antiretroviral therapy who had successful plasma viral suppression (HIV RNA level < 50 copies/mL) and baseline and follow-up CSF and neuropsychologic testing through the CHARTER (CNS HIV Anti-Retroviral Therapy Effects Research) study or the HIV Neurobehavioral Research Center (HNRC). Participants were classified as neurologically normal or as having neurocognitive impairment (ANI or MND) and were assessed for changes in neuropsychologic testing performance between 2 time points. CSF NFL levels were not elevated in the group of individuals with neurocognitive impairment at baseline (n = 70) or in those who had neurocognitive decline over the course of follow-up (n = 32). However, CSF neopterin was elevated in those with impairment at baseline compared with neurocognitively normal individuals, and was also elevated in the group that experienced neurocognitive decline. These data indicate that heightened intrathecal macrophage activation is associated with the presence and progression of impairment in participants on systemically successful treatment. These are the first data to tie a mechanism of neurologic injury to progressive clinical signs in well-treated individuals with HIV infection, and have important implications for strategies to reverse or ameliorate HAND.

Peluso and colleagues (Abstract 473) examined a novel immune activation marker assessed longitudinally in CSF before and after initiation of antiretroviral therapy in study participants with acute or chronic HIV infection in Thailand. CSF YKL-40, a systemic biomarker of inflammation and cancer that localizes to activated microglial cells and reactive astrocytes in the CNS, was measured in 33 individuals with acute HIV infection (median 18 estimated days of infection) compared with chronic infection (n = 34), owing to its predictive value in development of neurodegeneration (including SIV encephalitis). In individuals with acute HIV infection prior to intitiation of antiretroviral therapy, CSF YKL-40 was lower than in participants with chronic HIV infection and was not different than in HIV-uninfected Thai volunteers (n = 18). After initation of antiretroviral therapy during acute HIV

infection (6-month treatment duration), the median level of CSF YKL-40 remained stable and was statistically significantly lower than in those starting treatment during chronic HIV infection (12-month treatment duration). These findings suggest that microglial and perhaps astrocyte activation may be prevented or ameliorated by very early antiretroviral therapy.

Numerous studies presented at CROI 2015 examined how altered cellular bioenergetics or metabolism may contribute to neuropathogenesis of HAND. Two studies employed metabolomic profiling to assess processes associated with neurocognitive dysfunction. Haughey and colleagues (Abstract 497) explored how the composition of energy metabolites in the CSF, as measured by hydrogen nuclear magnetic resonance (^{1}H-NMR) spectroscopy, may relate to neurocognitive status over time in HIV-infected individuals. Using partial least squares regression, which allowed them to identify predictors associated with outcomes from a very large number of potentially contributing variables, these investigators found that the metabolites in CSF involved with aerobic glycolysis along with other clinical factors were predictive of neurocognitive decline, whereas those involved with anaerobic glycolysis were predictive of neurocognitive improvement. This pattern suggests potential novel approaches for predicting response to therapy in HIV-infected individuals. In a related study, Cassol and colleagues (Abstract 498) analyzed cellular metabolites in blood plasma using liquid or gas chromatography followed by mass spectrometry in 68 HIV-infected and 36 HIV-uninfected study participants. Participants were further classified by depression status based on a self-administered depression inventory. Depressed individuals had lower levels of metabolites of phenylalanine-tyrosine catabolism and acylcarnitine than individuals without depression. Interestingly, these results were found both in the HIV-infected and HIV-uninfected groups, suggesting that this pathway might be an important target for the treatment of depression regardless of HIV infection.

Alteration in mitochondrial function is postulated to potentially result from HIV infection itself and the toxic effects of antiretroviral medications. Funes and colleagues presented data from a study that investigated nitric oxide as a potentially crucial molecule in the mechanism of efavirenz-induced neuronal toxicity (Abstract 500). Levels of nitric oxide synthase and nitric oxide (a free radical associated with mitochondrial dysfunction and inflammation) were measured in human brain tumor cell lines and cultured rat cortical neurons after brief exposure to efavirenz. In this system, efavirenz provoked inducible nitric oxide synthase in glial cells, and this increase in nitric oxide impaired mitochondrial function. These effects were not seen in neurons, suggesting that bioenergetic toxicity of efavirenz may occur through glial dysfunction rather than neuronal injury. Therapies aimed at reducing nitric oxide or its precursors may reduce the potential adverse effects associated with efavirenz.

Another molecule that has been implicated in the etiology of HAND through induction of cell death and CNS inflammation is HIV transactivator of transcription (Tat). Brew and colleagues (Abstract 505) explored whether HIV Tat levels in CSF might remain persistently abnormal in individuals taking suppressive antiretroviral therapy, as Tat can be secreted by infected cells even during therapy. HIV Tat remained detectable in the CSF in 5 (13.5%) HIV-infected individuals taking antiretroviral therapy who had HIV RNA levels below 50 copies/mL in blood or CSF (n = 37). Detectable Tat was not associated with current HAND status or B-cell CLL/lymphoma 11B (zinc finger protein) (BCL11B), a putative measure of viral latency. However, further studies are needed to investigate whether levels of CSF Tat detected in individuals on successful systemic treatment may be associated with other highly sensitive markers of neuronal injury, such as CSF NFL or imaging markers of inflammation or neuronal injury.

Several studies focused on heme oxygenase-1 (HO-1) as a potentially neuroprotective factor in HIV-related brain injury. HO-1 has been previously shown to be reduced in the frontal cortex and striatum of HIV-infected individuals with HAD, and inversely related to HIV RNA level and neuroinflammation.[4] Gill and colleagues (Abstract 501) focused on the relationship between HIV strain and degree of macrophage HO-1 down regulation by studying an in vitro model of HIV-infected monocyte-derived macrophages (MDMs) isolated from noninfected donors and infected the cells with 15 HIV strains. In this study, replication of HIV strains consistently reduced HO-1 in MDMs. Levels of HO-1 were inversely associated with levels of viral replication and extracellular glutamate measured in supernatant in this MDM model, suggesting that enhancing production of HO-1 in MDMs may benefit the CNS in HIV.

HIV neuropathogenesis appears to relate to processes such as blood-CNS barrier disruption and intrathecal immune activation, which may persist despite initiation of antiretroviral therapy. Alteration of intracellular energy metabolism may be associated with neurologic or psychiatric morbidity and may be worsened by neurotoxic medications.

Duncan and colleagues (Abstract 502) presented a study that identified atorvastatin as a medication with potential benefit for the HO-1 deficiency noted in HIV infection. Atorvastatin belongs to a class of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that has numerous putative immunomodulatory effects, including reduction of monocyte or macrophage activation. Thus, the investigators used an in vitro model of primary MDMs infected with a macrophage-tropic HIV strain to assess the impact of atorvastatin treatment on HO-1. They found that although HIV infection of MDMs reduced HO-1, atorvastatin added to MDMs at 8 days postinfection increased HO-1 protein expression. This study not only

provides rationale for a targeted treatment study based on these preliminary findings, it also suggests a potential beneficial mechanism of broad statin use in ameliorating the neurologic processes associated with HAND.

Diagnosis of HAND

Key questions remain concerning how to identify HIV-infected individuals at increased risk for HAND. Rourke and colleagues (Abstract 465) assessed 575 HIV-infected adults taking antiretroviral therapy in the OCS (Ontario HIV Treatment Network Cohort Study). At baseline, participants had neuropsychologic performance testing and were categorized as normal (n = 299) or as having ANI (n = 276). At 52 weeks of follow-up a greater percentage of individuals with ANI progressed to more severe forms of HAND (MND or HAD) compared with the cognitively normal HIV-infected individuals. Factors associated with faster progression included depression and history of smoking.

Similar results were observed by Brouillette and colleagues (Abstract 469) in a subset of the CHARTER cohort (n = 191). Factors associated with cognitive decline included atherosclerotic vascular disease, duration of HIV infection, and education level. The investigators noted that a large proportion of the CHARTER cohort (approximately 80%) had modifiable risk factors, including smoking and a body mass index of 25 or higher. These risk factors could be targeted by primary care physicians.

The effects of potentially modifiable risk factors were further confirmed by several groups, using the VACS (Veterans Aging Cohort Study) Index score (risk points assigned for age, CD4+ cell count, plasma HIV RNA level, hemoglobin value, fibrosis stage, renal glomerular filtration rate, and presence of hepatitis C virus infection). Calcagno and colleagues (Abstract 487) demonstrated that HIV-infected participants with HAND (n = 441) were at higher cardiovascular risk based on the VACS Index. Rourke and colleagues (Abstract 467) showed that a higher VACS Index score at baseline was associated with greater cognitive decline (as assessed by neuropsychologic performance testing) at subsequent follow-up. Collectively, these results suggest that screening of higher-risk HIV-infected participants based on cardio- and cerebrovascular abnormalities (including smoking) may assist in the early diagnosis of HAND. Treating these modifiable risk factors could lead to a reduction in HAND.

Identification and treatment of modifiable risk factors (eg, smoking, obesity, or depression) could reduce HIV-associated neurocognitive disorders.

Neuroimaging

A variety of neuroimaging techniques were used to assess the effects of HIV in the CNS. Becker and colleagues (Abstract 494) used a trajectory model based on volumetric data from 3892 HIV-infected individuals followed by the MACS (Multicenter AIDS Cohort Study) to identify 3 possible trajectories of disease progression: 1) normal aging, a profile with relatively low probability of even mild impairment until middle age; 2) premature aging, a profile with the probability of mild impairment occurring at age 45 years to 50 years; and 3) unhealthy, a profile with a high probability of impairment at a young age. Changes in the posterior cingulate–precuneus cortex, the hippocampus, and the inferior frontal cortex were associated with the unhealthy profile, and changes in the cingulate gyrus, the insula, and the basal ganglia were associated with the premature aging profile.

Observed neuroimaging differences may assist in differentiating between the effects of HIV and those with aging. Sacktor and colleagues (Abstract 482) obtained ^{18}F-AV-45 positron emission tomography (PET) scanning to assess amyloid deposition in HIV-infected participants (n = 25) and HIV-uninfected controls (n = 6). HIV-infected individuals with symptomatic HAND (MND and HAD) had mildly increased amyloid depositions in the hippocampus and basal ganglia compared with HIV-infected individuals with ANI or normal cognition. Observed areas of increased amyloid deposition were different than those typically seen with Alzheimer's disease. However, amyloid depositions were not different for HIV-infected individuals compared with HIV-uninfected individuals.

Using another PET ligand, Vera and colleagues (Abstract 477) examined the relationship between microbial translocation (measured by 16S ribosomal [r] DNA) and brain inflammation ([^{11}C]–peripheral benzodiazepine receptor [PBR] 28) and structure (by diffusion tensor imaging [DTI]) in HIV-infected individuals (n = 12). An association existed between plasma 16S rDNA and brain biomarkers (^{11}C-PBR28 and DTI metrics). In addition, Smith and colleagues (Abstract 485) observed increased inflammation using a 3D postcontrast T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging technique. A greater proportion of HIV-infected participants had focal leptomeningeal contrast enhancement than did HIV-uninfected participants. Observed enhancement was not correlated with CD4+ cell count, CD4+ cell nadir, duration of HIV infection, or history of neurologic disorders. Finally, Cohen and colleagues (Abstract 935) demonstrated that perinatally HIV-infected children (n = 35) had statistically significant structural imaging (DTI and volumetric) and neuropsychologic performance differences compared with healthy, HIV-uninfected children (n = 37). These neuroimaging studies hint at the possibility of including imaging biomarkers in diagnostic criteria for HAND.

Novel neuroimaging markers may noninvasively assess structural and inflammatory changes seen in asymptomatic HIV-infected individuals, and thus may provide preclinical markers of and insight into pathogenesis of HIV-associated neurocognitive disorders.

Treatment of HAND

A number of different treatment options are now available and should

be considered for HIV-infected patients, especially those with HAND. Bowman and colleagues (Abstract 445) assessed HIV-infected, antiretroviral treatment–naive participants (n = 40) before and 2 weeks after initiating therapy. Viral load in blood decayed faster than in CSF after initiation of antiretroviral therapy. In general, HIV protease inhibitors were associated with faster CSF viral suppression, and integrase strand transfer inhibitors were associated with slower suppression.

There is increasing concern regarding the potential adverse effects of efavirenz on cognition. In a large Canadian cohort (n = 831), Rourke and colleagues (Abstract 448) observed no differences among HIV-infected participants who were currently taking efavirenz, those who had previously received efavirenz, or those who had never taken efavirenz. Although these results complement previous published studies nicely,[5,6] they conflict with other reports, including studies presented at CROI 2015 that suggest the potential neurotoxicity of efavirenz.

Ma and colleagues (Abstract 444) assessed the incidence of neurocognitive impairment in HIV-infected participants (n = 23) in China assigned an antiretroviral regimen of tenofovir, lamivudine, and efavirenz compared with a regimen of zidovudine, lamivudine, and nevirapine. A higher incidence of cognitive impairment was seen in HIV-infected participants receiving the regimen that included tenofovir than in those receiving the efavirenz-containing regimen. As part of the same randomized study, Letendre and colleagues (Abstract 56) presented results of these regimens used in antiretroviral treatment–naive, HIV-infected adults in China. Participants were randomly assigned to receive an open-label antiretroviral regimen of zidovudine, lamivudine, and nevirapine or tenofovir, lamivudine, and efavirenz, and were assessed longitudinally by neuropsychologic performance testing at 48 weeks and 96 weeks. The group taking the efavirenz-containing regimen had a greater risk of incident neurocognitive impairment than the group taking the nevirapine-containing regimen. However, those receiving the nevirapine-containing regimen had more adverse events than those receiving treatment containing efavirenz. For each of these studies, the investigators hypothesized that observed differences may reflect differences in drug distribution into the CNS or neurotoxicity.

With regard to other therapies currently being used to treat HIV-infected individuals with HAND, Caramatti and colleagues (Abstract 442) observed no differences in neuropsychologic performance in participants (n = 37) who received monotherapy with ritonavir-boosted (/r) atazanavir compared with those who received triple therapy that included atazanvir/r. For both groups, a substantial decrease in HAND was seen at 96 weeks. These results complement work by Ferretti and colleagues (Abstract 443) in a smaller nested cohort (n = 23), which showed that CSF viral escape was similar between HIV-infected participants receiving long-term, successful monotherapy with atazanvir/r and those receiving triple therapy that included atazanvir/r. Baker and colleagues (Abstract 447) demonstrated that the CNS penetration effectiveness (CPE) of antiretroviral therapy did not affect neuropsychologic performance and brain volumetrics in a cohort of HIV-infected participants (n = 64). No differences in neuropsychologic performance testing results or brain volumetrics existed between groups with low versus high CPE scores.

Gates and colleagues (Abstract 441) conducted a small randomized controlled trial among virologically suppressed HIV-infected participants with HAND (n = 19) who were assigned to receive antiretroviral therapy or antiretroviral therapy enhanced with maraviroc. Maraviroc was chosen because of its high level of CNS penetration and dual antiretroviral and anti-inflammatory activity. At 52 weeks, neuropsychologic performance had improved more in the maraviroc-containing arm than in the control arm, and neuroimaging measures of glutamate concentrations (a possible measure of excitotoxicity) were higher in the control arm than in the maraviroc-containing arm. Evering and colleagues (Abstract 446) studied individuals taking prolonged (median, 5.7 years) antiretroviral therapy that had been initiated at a median 1.6 months after infection, who did not have comorbidities such as depression or substance abuse. Cognitive impairment was observed in only 4% (1/26) of this group, suggesting the potential benefit of early treatment. Although many of these preliminary studies are promising, larger studies with longer follow-up are needed for HIV-infected participants with varying degrees of cognitive impairment.

Antiretroviral therapy enhancement with maraviroc may lead to reduced inflammation and better neurocognition. However, larger longitudinal studies are needed.

Adjunctive measures (eg, exercise and engagement in mental exercises) were also considered for HIV-infected participants at risk for cognitive disorders. Basco and colleagues (Abstract 488) studied neuropsychologic performance, neuroimaging, and self-reported aerobic exercise in HIV-infected individuals categorized as physically active (n = 22) or sedentary (n = 48). Physically active participants performed statistically significantly better than sedentary HIV-infected participants on neuropsychologic performance tests of executive function but not of motor function. Monroe and colleagues (Abstract 489) found similar results in the large MACS cohort of HIV-infected men (n = 622). High physical activity was associated with better neuropsychologic performance on executive and psychomotor tests than was low physical activity. Whether exercise leads to cognitive improvement or whether cognitive improvement leads to an increased ability to participate in exercise remains in question.

Pinnetti and colleagues (Abstract 63) demonstrated that in a single-site cohort (n = 569), better virologic control (higher current CD4+ cell count and lower viral load) and higher education level were associated with reduced

risk of developing HAND in the current antiretroviral therapy era. Results from Milanini and colleagues (Abstract 495) showed that in a cohort of 50 HIV-infected participants, higher cognitive reserve (assessed by IQ) was associated with lower risk of ANI. Overall, these studies suggest that engagement in physical, intellectual, and social activities may independently protect against cognitive impairment in HIV-infected individuals, but larger studies with more detailed measurements of physical function and cognitive reserve before and after an intervention are needed.

Adjunctive measures (including engagement in physical and mental exercises) may help reduce HIV-associated neurocognitive disorders. However, larger longitudinal studies are needed.

New Frontiers for Understanding HAND and HIV Neuropathogenesis: Resource-Limited Settings

At CROI 2015, neurologic studies conducted in resource-limited settings, led by or in collaboration with local investigators were emphasized. Kambugu and colleagues (Abstract 57) presented data from the EARNEST (Europe-Africa Research Network for Evaluation of Second-Line Therapy) study examining the magnitude of and factors associated with neurocognitive function at the time of failure of initial antiretroviral therapy, and assessing any changes in neurocognitive function that may occur after switching antiretroviral treatment. The EARNEST trial enrolled 1277 participants whose initial antiretroviral therapy had failed according to World Health Organization clinical and immunologic criteria at the time of the study design. Individuals were randomly assigned to receive lopinavir/r plus 2 or 3 nucleotide analogue reverse transcriptase inhibitors; a protease inhibitor plus raltegravir; or protease inhibitor mono therapy (with a 12-week raltegravir induction period). Three domains were examined by neurologic testing at baseline, week 48, and week 96: the Color Trails Test 1 (measuring attention and concentration), the Color Trails Test 2 (measuring cognitive flexibility), and the Grooved Pegboard test (measuring psychomotor speed/fine motor skills). Test scores were standardized to a *z* score based on demographically adjusted, US-derived norms and were then averaged into an overall score (NPZ-3 score). Mean composite *z* score at baseline was -2.96, suggesting that individuals in this study had performance levels approximately 3 standard deviations below the norm at baseline. In multivariable analyses, *z* scores were independently lower with a number of factors, including older age, lower body weight, higher viral load, lower hemoglobin value, and fewer years of education. Scores improved substantially after starting antiretroviral treatment, with equal improvement between regimen arms. The dramatically low *z* scores of individuals in the EARNEST study at baseline may reflect true substantial cognitive impairment or may indicate that norms derived from the United States may not yield accurate results when assessing individuals in resource-limited settings.

A number of other studies detailed the prevalence or incidence of HAND, or response to treatment in resource-limited settings. Robertson and colleagues (Abstract 451) presented the results of the AIDS Clinical Trials Group (ACTG) 5199 (International Neurological Study) and the ACTG 5271 (International Neurocognitive Normative Study) studies. These large studies accrued more than 3200 participants over 12 years in various countries (Malawi, Thailand, Brazil, India, Peru, Zimbabwe, and South Africa), specifically included site-specific HIV-uninfected individuals appropriately matched to HIV-infected articipants, and performed extensive coordinated oversight of the sites involved in the study for quality control. These studies demonstrated that the prevalence of neurocognitive impairment compared with well-matched HIV-uninfected controls is similar to what is seen in the United States: 25% with mild impairment, 17% with moderate impairment, 3% with severe impairment, and 54% falling in the normal range. Effective antiretroviral therapy reduced neurocognitive impairment substantially over time, from 46% at baseline to 28% at week 168 of treatment.

Sacktor and colleagues (Abstract 452) conducted a large study of HIV-infected (n = 299) and HIV-uninfected indiviuals (n = 210) living in rural Rakai, Uganda, and found a very high rate of HAD in antiretroviral treatment–naive individuals compared with normative data from HIV-uninfected individuals in Kampala, Uganda. Twenty-seven percent of individuals with HIV infection met criteria for HAD compared with 7% of HIV-uninfected individuals. Most HIV-infected individuals in this region harbor HIV subtype (or clade) A or D, allowing for important future analyses regarding possible associations between HIV subtype and risk for HAND. Finally, Valcour and colleagues (Abstract 459) studied more than 900 participants from East Africa in order to investigate determinants of neuropsychologic performance in the AFRICOS (The African Cohort Study) study. Cognitive impairment, as measured by all testing methods, was associated with HIV infection status, age, and level of education. Nadir CD4+ cell count was also associated with performance on 2 specific tests. However, cognitive performance was not associated with number of infectious and noninfectious comorbidities, a finding distinct from studies in resource-endowed settings. All of these studies highlight the need for appropriate norms to study correlates to neurocognitive disorder that are relevant in resource-limited settings, and the need to collaborate with and foster investigations by local experts in order to implement highly rigorous and relevant research in these settings.

Financial affiliations in the past 12 months: Drs Spudich and Ances have no relevant financial affiliations to disclose.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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Review

CROI 2015: Complications of HIV Infection and Antiretroviral Therapy

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Noncommunicable diseases, such as cardiovascular disease, hypertension, renal and bone disease, and malignancies are an ongoing concern during the course of treated HIV disease. Research in this area continues to focus on the epidemiology and risk factors associated with these conditions, identifying the contributions of HIV-related immunopathology to specific and collective end-organ diseases, and evaluating interventions to prevent or reduce the morbidity associated with these conditions. Infectious complications of HIV, such as tuberculosis and cryptococcal disease, also continue to cause substantial morbidity and mortality; diagnosis, prevention, and treatment of these is an area of focus. The 2015 Conference on Retroviruses and Opportunistic Infections provided new insights into all of these areas.

Keywords: CROI 2015, complications, HIV, cardiovascular disease, comorbidities, statins, bone, renal, pulmonary, malignancy, tuberculosis, opportunistic infections, cryptococcal meningitis

Burden of Disease

At the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 23 to 26, trends in age-adjusted rates of hypertension, diabetes, and chronic kidney disease were examined by Wong and colleagues from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) (Abstract 1053). Rates of all 3 conditions increased with each decade after age 40 years, with the highest rates among blacks of all ages and among older adults. These findings underscore the importance of primary care services for all adults with HIV infection.

Chronic noncommunicable diseases are not limited to people residing in high-income settings. In addition, the excess risk of these conditions that is attributable to HIV disease has not been well studied in low- and middle-income settings. In the massive, multidisease screening campaign SEARCH (Sustainable East Africa Research of Community Health) underway in Uganda, blood pressure measurements were obtained for 65,274 adults from 20 rural communities. The overall prevalence of hypertension was 15.6% after age standardization. Expected associations between age, male sex, higher body mass index (BMI), and alcohol use and higher risk of hypertension were observed. Notably, the adjusted relative odds ratio of hypertension was 1.2 times higher for HIV-seronegative than -seropositive individuals. Among the group with HIV infection, the rate of hypertension was 10.2%; viral suppression did not predict hypertension, and slightly more than half achieved control of blood pressure with treatment. These results suggest that HIV may not contribute to an excess risk for hypertension.

As highlighted above, traditional risk factors contribute most to the risk for hypertension, and the role of specific antiretroviral drugs as contributors to hypertension remains controversial. D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study investigators examined data on more than 30,000 people to address this question, and they confirmed that established risk factors rather than antiretroviral drugs predict hypertension risk (Abstract 739). Regardless of whether HIV infection augments hypertension risk, optimal strategies for treatment of hypertension for those with HIV infection will still be needed. Among 10-year survivors taking antiretroviral therapy who were followed up in Haiti, 25% had 1 or more noncommunicable diseases (58% hypertension, 3% diabetes, and 39% chronic lung disease) (Abstract 156).

Inflammatory Biomarkers and End-Organ Disease and Mortality

Elevations in interleukin (IL) 6, soluble CD14 (sCD14), and d-dimer levels are prevalent during treated HIV disease and have been shown to be strong predictors of mortality. An analysis done by Veterans Affairs investigators examined mortality in a group of HIV-infected individuals compared with -uninfected controls, to attempt to delineate the contributions of these biomarkers and their independence from HIV RNA level in predicting mortality. After adjusting for age, race, and other comorbidities, HIV RNA levels and these biomarkers (IL-6, sCD14, and d-dimer) remained independent predictors of mortality. These results support the hypothesis that inflammation contributes to mortality in HIV infection (Abstract 1049). The relative strength of the association between

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IL-6, high-sensitivity C-reactive protein (hsCRP), and d-dimer and clinical events was further investigated in a study combining data from the control arms (those who received only antiretroviral therapy) of the SMART (Strategies for Management of Antiretroviral Therapy) and ESPRIT (Evaluate Recombinant Interleukin-2 in HIV-Positive Patients Taking Antiretroviral Therapy) trials (Abstract 761). Independently, IL-6 level was a stronger predictor of mortality than other measures, especially for non-AIDS mortality, whereas d-dimer level was a stronger predictor of progression to AIDS. Whether interventions directed at reducing IL-6 level will alter the course of HIV disease remains to be shown.

sCD14 is a measure of monocyte activation that has been associated with all-cause mortality and progression of atherosclerosis in treated HIV infection.[1,2] Previous studies have suggested that daily acyclovir use reduced the risk of progression of HIV infection; however, the mechanism is unclear. Using stored samples from earlier studies of acyclovir in HIV-infected women, researchers identified that acyclovir use was associated with faster declines in sCD14, suggesting that a reduction in monocyte activation may be the mechanism of action of the acyclovir effect previously observed (Abstract 321). sCD163, a scavenger receptor expressed by monocytes and macrophages that is shed when these cells are activated, has been linked to vascular disease risk in HIV-infected and -uninfected patients.[3] Cytomegalovirus (CMV) coinfection is a candidate mediator of the excess inflammation observed during treated HIV infection. Investigators from the ICONA (Italian Cohort of Antiretroviral Naive Patients) study examined the relationship between CMV coinfection and measures of inflammation, noting that those with CMV coinfection had higher levels of sCD163, whereas although sCD14 levels were higher in the coinfected group, the difference did not reach statistical significance (Abstract 303). They also noted a strong correlation between the level of sCD163 and anti-CMV immunoglobulin G (IgG) levels. A correlation between anti-CMV IgG and higher levels of sCD14 was also noted, in another report comparing patients with CMV-associated end-organ disease with controls. (Abstract 302) These findings add to growing literature suggesting that persistent CMV infection may be an important driver of inflammation in treated HIV disease.

MACS (Multicenter AIDS Cohort Study) investigators used an exploratory factor analysis to examine the relationship between a panel of biomarkers of inflammation, in an attempt to sort out the underlying inflammatory pathways that contribute to decline in renal function among treated and virologically suppressed individuals. sCD14 emerged among the group of measures (including soluble tumor necrosis factor receptor 2 [sTNF-R2], sIL-2 receptor, soluble glycoprotein [sgp]130, and sCD27) that were associated with a decline in kidney function, whereas IL-6, IL-8, and TNF-α were not associated with this outcome (Abstract 797).

Whether antiretroviral therapy regimens differ in their impact on biomarkers of inflammation during initial treatment remains an open question. Hileman and colleagues reported that the combination of elvitegravir, cobicistat, emtricitabine, and tenofovir led to a greater decrease in sCD14 and lipoprotein-associated phospholipase A2 [Lp-PLA2] levels over a period of 48 weeks than did efavirenz, emtricitabine, and tenofovir; no differences were noted in the impact of these regimens on IL-6, sTNF-R1, or sCD163 levels (Abstract 738). The clinical significance of the magnitude of the decline in sCD14 level (approximately 10%) in the group that received elvitegravir is unknown but warrants longer-term follow-up.

Cardiovascular Disease

Pathogenesis of Cardiovascular Disease in HIV

Two elegant studies examined the pathogenesis of cardiovascular disease (CVD) in HIV infection using animal models and human tissue samples. Panigrahi and colleagues used the simian immunodeficiency virus (SIV) rhesus macaque model to examine the role of endothelial factors in athero sclerosis. In this study, investigators compared paraffin-embedded slides from the aortas of SIV-infected and -uninfected animals with staining for the endothelial factor Krüppel-like factor 2 (KLF2), a transcriptional master regulator that promotes an antithrombotic endothelial environment and endothelial nitric oxide synthase (eNOS) (Abstract 298LB). They observed focal endothelial proliferation and infiltration of monocytes, T lymphocytes, and platelets in 3 of the 4 SIV-infected animals, but in none of the controls. They also found reduced levels of eNOS and KLF2 in the SIV-infected group. Further, in experiments using cultured primary endothelial cells, the investigators noted that simvastatin could protect against the down regulation of KLF2, further supporting a potential beneficial role for statins in preventing CVD in the setting of HIV infection.

Walker and colleagues, using human tissue samples of the aorta and left ventricle in 10 HIV-seronegative and 10 -seropositive individuals with CD4+ counts less than 200 cells/μL, reported an increased number of macrophages in aortic tissue in the HIV-seropositive group compared with controls, and a strong correlation between increased numbers of CD163+, CD68+, MAC387+, and CD206+ macrophages and the percentage of collagen (fibrosis) in ventricular tissue in the HIV–seropositive group (Abstract 753). They also deployed the novel radioisotope tilmanocept in a probe that binds to CD206+ and CD163+ macrophages to demonstrate the presence of these macrophages in tissue sections. This probe is currently used as a diagnostic imaging agent in vivo in patients with head and neck cancers. These results suggest that tilmanocept could potentially have a role in the assessment of macrophage-related vascular inflammation in individuals with HIV infection.

Another intriguing area of investigation is the links between microbial translocation, the gut microbiome, and CVD risk in HIV infection. Srinivasa and colleagues examined the relationship between microbial-derived, choline-related metabolites (trimethylamine [TMA] and trimethylamine N-oxide [TMAO]) and coronary plaque using stored samples from an earlier study of computed tomography angiography (Abstract 138). They found that TMA, a microbial-derived precursor of TMAO, was associated with the number of total and calcified plaque segments, and with calcium plaque volume in HIV-infected participants.

These associations were only noted in the HIV-infected group and not in the control group, prompting speculation about the role of altered gut flora in the setting of HIV infection. Sinha and colleagues from University of California San Francisco also investigated the link between TMAO and atherosclerosis in patients with HIV infection (compared with HIV-uninfected controls) and coronary artery disease using carotid intima-media thickness (CIMT) as the measure of atherosclerosis (Abstract 755). In this study, TMAO levels were similar in the younger HIV-infected group and the older group of HIV-uninfected patients with known coronary artery disease, and appeared to be associated with antiretroviral therapy in the HIV-infected group. An association between higher TMAO levels and CIMT that weakened after adjustment was noted. Further study of this area is expected in the coming years.

Statins

Several studies examined the impact of statins on surrogate measures of atherosclerosis. Lo and colleagues reported data from a small randomized controlled trial in which atorvastatin reduced noncalcified coronary plaque volume and other features of high-risk plaque when compared with placebo (Abstract 136). McComsey and colleagues reported 96-week follow-up data from SATURN-HIV (Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV), a study that compared rosuvastatin with placebo among patients with HIV infection and low-density lipoprotein (LDL) cholesterol levels below 130 mg/dL and baseline elevation in T-cell activation (Abstract 137). CIMT progressed measurably over 96 weeks in the placebo group but not in the group that received rosuvastatin. No statistically significant differences in coronary calcium were noted.

Another important finding from the SATURN-HIV study was that higher levels of self-reported activity (eg, exercise) were associated with lower levels of inflammatory biomarkers at baseline and with improved measures of vascular health during study follow-up (CIMT, carotid distensibility, and flow-mediated dilation) (Abstract 745).

There is growing evidence of the potential benefits of statins in HIV treatment.

Statins may have benefits beyond reducing the risk of CVD in the setting of HIV disease. Nakanjako reported the results of a small randomized study comparing atorvastatin with placebo in patients taking antiretroviral therapy in Uganda in whom CD4+ cell count had not increased. During 12 weeks of follow-up a reduction in CD4+ and CD8+ T-cell activation was noted in the group that received atorvastatin (Abstract 322) (see also Nakanjako et al, 2015[4]).

A common question in clinical practice is whether patients who develop hypercholesterolemia while taking a ritonavir-boosted protease inhibitor (PI/r) should switch their antiretroviral regimen or add a statin drug. The answer depends in part on the PI/r involved and the specific lipid abnormality. Lee and colleagues enrolled virologically suppressed patients taking a PI/r with a total cholesterol of at least 5.5 mg/dL and an elevated Framingham risk score (FRS) to randomly add rosuvastatin 10 mg per day or switch the PI/r to another drug (raltegravir or rilpivirine). After 12 weeks of follow-up, those randomized to receive rosuvastatin had statistically significantly greater declines in levels of total cholesterol and LDL cholesterol (-29.0% vs -1.0%, respectively), and ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol, whereas triglyceride levels improved more in those who switched PI/r (-34.0% vs -9.8%, respectively) (Abstract 733). These results confirm that for improvement of LDL cholesterol level, the addition of a statin is likely to be more beneficial than a switch in antiretroviral regimen.

Predicting CVD Risk

Accurate estimation of CVD risk can help to prioritize patients for interventions aimed at reducing disease. Previous studies have documented the underestimation of CVD risk using FRS, leading to the development of an HIV-specific risk prediction rule by the D:A:D study group. Several groups reported studies examining the performance of the new pooled-risk equation included in the 2013 American College of Cardiology (ACC)/American Heart Association (AHA) Guideline on the Assessment of Cardiovascular Risk.[5]

These studies were showcased during a themed discussion session chaired by Friis-Møller from the D:A:D study group (Session TD-P; Abstracts 747, 750, and 751). Using established cohorts of different sizes with different lengths of follow-up, results across these studies were remarkably consistent and demonstrated that although the new pooled-risk equation identifies more HIV-infected patients as having high risk than does FRS, it still underpredicts the rate of events. When Systematic Coronary Risk Evaluation (SCORE) and D:A:D pooled-risk equations were examined, the accuracy of estimation did not further improve, suggesting that further refinement of CVD risk prediction in the setting of HIV disease is warranted.

Currently available risk prediction scores likely underestimate CVD risk in the context of HIV infection.

In the same session, investigators from NA-ACCORD reported on the incidence of and risk factors for primary (atherosclerotic) disease or secondary myocardial infarction (MI; supply-demand mismatch) in a cohort of 25,000 participants with more than 100,000 person-years of follow-up and central ascertainment, adjudication, and classification of MI by type. The incidence rates for primary and secondary MI were similar, whereas the risk factors for each type varied.

Sepsis and cocaine use were seen in 50% of secondary MIs, whereas HIV RNA level above 400 copies/mL, time-updated CD4+ cell count, and history of an AIDS-defining illness were risk factors for primary MIs. These findings highlight the importance of managing HIV disease–specific risk factors in order to reduce rates of primary MI, and of the importance of cocaine use in MI risk.

Abacavir and MI Risk

The anticipated results from the NA-ACCORD cohort analysis examining the relationship between abacavir exposure and MI risk were presented at CROI 2015. With more than 16,733 adults in follow-up and 301 incident MIs, it was once again found that those who initiated treatment with abacavir had a higher prevalence of traditional and nontraditional HIV-related risk factors (eg, detectable HIV RNA, history of injection drug use) for MI. After adjusting for traditional risk factors (similar to D:A:D analyses), an association between abacavir and MI risk persisted (adjusted hazard ratio [aHR], 1.71; 95% confidence interval [CI], 1.11, 2.64). However, when the HIV-specific, nontraditional risk factors that were present prior to abacavir use were included in the model, the association was no longer statistically significant (aHR 1.34; 95% CI, 0.96, 1.88). Although this may not be the final word on the association between abacavir and MI, it does add credence to the notion that for people with established risk factors for CVD, alternatives to abacavir should be considered (Abstract 748).

Dyslipidemia

Proprotein convertase subtilisin/kexin 9 (PCSK9), an enzyme involved in lipid metabolism, is a new target for lowering LDL cholesterol levels using monoclonal antibodies that target this pathway (see also Robinson et al, 2015[6]). Little was known about PCSK9 levels in HIV-infected patients prior to the study reported by Kohli and colleagues from University of California San Francisco at CROI 2015. PCSK9 levels were 10% higher in the HIV-infected group than in the control group, after adjusting for demographics, statin use, and CVD risk factors. (Abstract 731). Whether this new class of lipid-lowering agents will have a role in the setting of HIV disease remains to be seen.

Antiretroviral therapy–induced dyslipidemia has been well described around the globe, but there have been fewer data from low- and middle-income settings, especially among children. Innes and colleagues reported on the prevalence of dyslipidemia and insulin resistance among a cohort of 100 perinatally HIV-infected children from South Africa who were receiving antiretroviral therapy. (Abstract 929). Although the prevalence of individual abnormalities (insulin resistance, total cholesterol, LDL cholesterol, and HDL cholesterol) occurred in less than 15% of the population, 40% of the children overall had at least 1 lipid abnormality or insulin resistance, suggesting that monitoring for early onset vascular disease may be warranted if treatment with current antiretroviral drugs (lopinavir/r and efavirenz) continues long term.

Other somewhat reassuring studies of children highlighted the use of pulse wave velocity as an easy, noninvasive way to measure CVD risk that correlates with CIMT (Abstract 925). In addition, among a group of HIV-infected, school-age children taking antiretroviral therapy in South Africa, pulse wave velocity did not appear altered compared with that of HIV-uninfected controls (Abstract 926).

Fat

The impact of initial and second-line antiretroviral therapy on body fat and inflammation are best evaluated using data from randomized trials. Erlandson and colleagues reported on the relationship between BMI, weight gain, and measures of inflammation in a random sample of adults (50% women) participating in the AIDS Clinical Trials Group (ACTG) A5175 study of antiretroviral therapy in diverse settings (Abstract 778). Changes in biomarkers of inflammation varied depending on baseline BMI. Among those who were underweight, initiation of antiretroviral therapy was associated with decreased hsCRP and stable or declining sCD14, whereas among those who were normal weight or overweight at baseline, increases in these markers were noted. These findings underscore the possible contributions of preexisting obesity to biomarker elevations during antiretroviral therapy.

The SECOND-LINE study evaluated a nucleoside analogue reverse transcriptase inhibitor (nRTI)-containing (lopinavir/r plus 2 nRTIs) and an nRTI-sparing (lopinavir/r plus raltegravir) regimen for patients whose initial antiretroviral regimen with an NNRTI plus an nRTI had failed (note: in this study, 48% of participants had prior thymidine nRTI exposure in their initial regimen, including stavudine). The study demonstrated the noninferiority of the nRTI-sparing combination, with respect to virologic suppression, with greater preservation of bone mass in the nRTI-sparing arm (Abstract 779). In a subanalysis of this trial using dual-energy x-ray absorptiometry (DEXA) scanning, the investigators hypothesized that the nRTI-sparing arm would be associated with greater gains in limb fat over 96 weeks of follow-up; however, after adjusting for covariates associated with limb fat loss, no difference was observed. These findings indicate that limb fat loss does not appear to be a progressive issue when thymidine nRTI exposure is minimized.

Metabolic changes were also examined in the 2LADY (Agence Nationale

de Recherches sur le Sida et les Hépatites Virales [ANRS] 12169) trial, which compared 3 regimens: lopinavir/r, tenofovir, and emtricitabine; abacavir, didanosine, and lopinavir/r; and tenofovir, emtricitabine, and darunavir/r (Abstract 782). Notably, 71% of study participants were women and 32% of study participants were overweight or obese at baseline. Over 48 weeks of follow-up, weight gain was greater in the group receiving the darunavir/r-containing regimen (>25% of participants had BMIs that increased from normal range to overweight range), whereas more abnormalities were noted among those who received abacavir and didanosine in the regimen, confirming the lack of enthusiasm for this regimen as a current treatment option.

A misconception persists that PIs are more likely to lead to central fat gain than other classes of drugs, despite data indicating the contrary from randomized trials and cohort studies.[7] The integrase strand transfer inhibitor raltegravir has negligible metabolic effects; however, its impact on visceral fat gain was not previously evaluated in well-powered studies.

McComsey presented the results of the ACTG 5260s study, which randomized HIV-infected, treatment-naive individuals to receive tenofovir and emtricitabine combined with one of atazanavir/r, darunavir/r, or raltegravir (Abstract 140). After 96 weeks of follow-up, the median percentage increases in limb fat, subcutaneous adipose tissue, visceral adipose tissue, trunk fat, and lean mass were observed in all groups (all participants gained fat in each of these areas) and did not differ between arms. Notably, the mean percentage increase in visceral adipose tissue was 31%, 33%, and 29% for those randomized to receive atazanavir/r, raltegravir, and darunavir/r, respectively.

These findings suggest that central fat gain after initiation of antiretroviral therapy is independent of the regimen selected. Further work is needed to determine whether these gains in fat have adverse health consequences or if they represent a "return to health" among patients being treated for chronic HIV infection.

Bone

There continues to be considerable interest in the study of bone disease in the setting of HIV infection, with studies presented at CROI 2015 focused on risk factors for bone loss, strategies to identify patients at risk for this complication, and interventions aimed at reducing bone loss. Confirming previous observations, it was again found that measures of inflammation, immune activation (including monocytes expressing tissue factor), and replicative senescent T cells were predictive of bone loss (Abstract 772). In addition, a previous report[8] suggesting that rosuvastatin protected against bone loss at 48 weeks was updated this year, and indicated that this association was no longer present at 96 weeks (Abstract 771).

The Fracture Risk Assessment Tool (FRAX) calculator may underestimate fracture risk in HIV-infected patients.

Antiretroviral therapy use and HIV infection contribute to bone loss and fracture risk. The web-based Fracture Risk Assessment Tool (FRAX) has been used to help stratify patients for interventions to reduce bone loss, yet the performance of this tool among those with treated HIV infection is unclear. Yin and colleagues used data from the Veterans Aging Cohort Study to test the performance of the modified FRAX (without available bone mineral density [BMD]) in 26,000 HIV-seropositive and -seronegative men aged 50 years to 70 years with available data and follow-up information (Abstract 141). FRAX underestimated fracture rates more frequently in the HIV-seropositive than in the -seronegative group.

When secondary osteoporosis was included as a risk factor, the performance of the FRAX calculator improved for the HIV-seropositive group. When thresholds for pharmacologic intervention were evaluated, FRAX had a poor predictive value of for identifying men who might benefit from therapy. These results suggest that modification of the existing risk calculator may improve the performance of this tool for men; however, further work is needed to determine the most appropriate threshold for intervention.

Tenofovir disoproxil fumarate (tenofovir) use is associated with bone loss of approximately 1% to 2% in the first few years of exposure. The newer investigational drug tenofovir alafenamide fumarate (TAF) has 90% lower plasma levels than tenofovir, with the prospect of lower bone effects. Sax presented the late-breaking bone outcome results of 2 combined phase III randomized trials comparing elvitegravir, cobicistat, emtricitabine, and tenofovir with elvitegravir, cobicistat, emtricitabine, and TAF over 48 weeks (Abstract 143LB). The reductions in bone density at the hip (-3.26% vs -0.66%, respectively; $P < .001$) and the spine (-2.86% vs -1.3%, respectively; $P < .001$) were significantly lower in the group receiving TAF than in the group receiving tenofovir, and increases in markers of bone turnover were less in the former group. These results confirm data from smaller earlier studies suggesting that TAF will have a more favorable bone safety profile than tenofovir.

Another approach to preventing bone loss during antiretroviral therapy is the use of tenofovir-sparing regimens. Taiwo and colleagues presented the results of a 48-week randomized trial comparing darunavir/r and emtricitabine combined with maraviroc 150 mg or tenofovir 300 mg. The regimens were comparable in terms of virologic outcomes, whereas declines in BMD were greater in tenofovir recipients (total hip BMD -1.51 in the group receiving maraviroc vs -2.40 in the group receiving tenofovir; $P < .001$; and lumbar spine BMD -0.88 in the group receiving maraviroc vs -2.35 in the group receiving tenofovir; $P < .001$) (Abstract 769LB).

Replacing tenofovir with abacavir has previously been shown to improve bone loss. Negredo and colleagues reported measures of bone turnover in the follow-up to an ongoing switch

study in which tenofovir was replaced with abacavir (Abstract 767). During treatment with abacavir, levels of C-terminal telopeptide of collagen type 1, osteocalcin, and procollagen type 1 N-terminal propeptide fell, whereas they remained unchanged during treatment with tenofovir. In addition, levels of sclerostin rose in the group receiving abacavir. Sclerostin, a protein produced by osteocytes that inhibits bone formation, is the target of a novel class of drugs for the treatment of osteoporosis.

Renal Disease

There is continued interest in the long-term renal effects of antiretroviral therapy. Although initial declines in renal function have been observed with some drugs, it has been less clear whether this continues to progress over time as a cumulative effect. Investigators from the D:A:D study group reported the incidence of chronic kidney disease—defined as a decline in estimated glomerular filtration rate (eGFR) from greater than 90 mL/min/1.73 m^2 to less than 60 mL/min/1.73 m^2—among 23,560 participants with normal renal function at baseline followed up for a median of 6.3 years (Abstract 142). Chronic kidney disease developed in 0.9% of the participants, and rates were increased for those exposed to tenofovir, lopinavir/r, and atazanavir/r but not for those exposed to abacavir or other boosted PIs. Notably, the decline in renal function occurred immediately after starting antiretroviral therapy and persisted with increasing durations of exposure. After discontinuing treatment, renal function improved only for those taking tenofovir.

In another presentation from the D:A:D study group, the link between renal impairment and risk for CVD was confirmed. Among HIV-infected individuals with a baseline eGFR of greater than 90 mL/min/1.73 m^2, the risk of incident CVD was 1.7%, compared with a 23.4% risk among those with an eGFR of up to 30 mL/min/1.73 m^2 (Abstract 742).

Pulmonary Disease

Studies examining the pulmonary complications of HIV disease at CROI 2015 included studies from African settings and studies of children. Risk factors for airway obstruction in Kenyans with HIV infection included vertically acquired HIV infection and, not surprisingly, cigarette smoking. No association was noted between CD4+ cell count, antiretroviral therapy use, or biofuel burning (Abstract 800). In the PHACS/AMP (Pediatric HIV/AIDS Cohort Study/Adolescent Master Protocol) study, pulmonary function of perinatally HIV-infected youth was compared with that of HIV-exposed, -uninfected youth in various US cities (Abstract 801). The rate of pulmonary function abnormalities was similar between the groups. However, the HIV-infected group had a lower rate of reversibility of airway function after use of bronchodilators, suggesting that respiratory symptoms in perinatally HIV-infected youth might be misclassified as asthma. Finally, Morris and colleagues examined the relationship between lung function and cognitive impairment in a subgroup of men who had measures of each and observed that lower diffusion capacity was associated with a worse cognitive summary score, independent of HIV infection (Abstract 490).

Malignancies

Observational studies continue to shed light on the epidemiology of malignancies in the era of antiretroviral therapy and aging HIV-infected patients. Yanik conducted a case-cohort study of 5% of US Medicare enrollees and all cancer cases among persons aged 65 years or older (Abstract 725). Over 1- or 5-year periods, 2.5% and 10%, respectively, of persons aged 65 years or older were diagnosed with cancer. HIV-infected persons had a higher risk for Kaposi sarcoma and Hodgkin lymphoma than HIV-uninfected persons. In this study, HIV infetction was associated with lower risk for prostate cancer. Althoff and colleagues evaluated the population-attributable fraction for smoking and HIV-related risk factors for non–AIDS-defining cancers (NADCs) among adults in a North American cohort (Abstract 726). Among 39,554 adults, investigators identified 592 incident cancers; most frequent were lung cancer (17%), anal cancer (16%), prostate cancer (10%), Hodgkin lymphoma (9%), liver cancer (7%), and breast cancer (7%). Smoking was the most powerful risk factor for NADCs. HIV immunosuppression was also a detectable risk factor for NADCs. NADC risk was 34% higher for individuals with CD4+ counts below versus above 200 cells/μL (aHR, 1.34; 95% CI, 1.11-1.62). Hepatitis B virus infection was associated with a 64% increased risk for NADCs. The investigators estimated that smoking cessation programs starting in adolescence could prevent as many as 46% of NADCs in HIV-infected adults. Effective antiretroviral therapy could prevent 6% of NADCs. At the other end of the age spectrum, Bohlius and colleagues evaluated AIDS-related and non–AIDS-related cancers among HIV-infected children aged 16 years or younger in several South African cohorts (Abstract 724). Kaposi sarcoma and non-Hodgkin lymphoma were the most frequent cancers, and there were few NADCs. As expected, the overall risk for cancer was lower among children treated with antiretroviral therapy.

Tuberculosis and Cryptococcal Disease

Tuberculosis

Diagnosis. In 2010, the World Health Organization (WHO) recommended a rapid combined tuberculosis (TB) and resistance to rifampicin assay (Xpert MTB/RIF) as the first-line diagnostic test for individuals suspected of having HIV-associated TB or multidrug-resistant TB. South Africa, which has the highest burden of HIV/TB coinfection in the world, rapidly adopted this recommendation. One challenge that arose from adoption of this new assay was related to assay performance. The sensitivity of the assay—although greater than that of an acid-fast bacilli

(AFB) smear—remained less than that of a TB culture, and this appeared to be a limiting factor in terms of its impact on clinical decision making. The lack of data on assay performance in a settings with lower TB prevalence have also limited widespread use of the assay in places such as the United States. Two presentations at CROI 2015 provide support for wider use of this remarkable technology.

The standard Xpert MTB/RIF assay has the potential to rule out TB that requires isolation, in addition to providing faster confirmation of TB diagnosis. In other words, hospitalized patients with suspected TB that are initially placed in isolation rooms could be moved to standard rooms based on the results of this rapid test. Current Centers for Disease Control and Prevention guidelines call for serial negative AFB smear results before patients with suspected TB can be removed from isolation.[9] Luetkemeyer and colleagues compared the sensitivity and specificity of 2 Xpert MTB/RIF tests with 2 AFB smears among 633 patients with suspected TB (Abstract 824). Thirty-eight percent of these patients were HIV infected. The gold standard was a TB culture. As expected, the 2 Xpert MTB/RIF assays were more sensitive than the 2 AFB smears in detecting TB (85.2% vs 69.3% sensitivity, respectively). Two Xpert MTB/RIF tests detected all AFB smear–positive TB-infected patients, thus identifying all patients requiring isolation based on sputum AFB smear. Based on these and other data, the US Food and Drug Administration approved the Xpert MTB/RIF assay in 2015 as a tool for decision making regarding respiratory isolation for patients with suspected TB.[10]

The Xpert MTB/RIF assay can be used for decision making with regard to respiratory isolation of TB-infected patients.

At one of the most exciting presentations at CROI 2015, Alland and colleagues presented data on a new and improved Xpert MTB/RIF assay "with sensitivity equal to culture" (Abstract 91). The Xpert MTB/RIF assay optimization included 3 key elements: 1) an increase in the size of the DNA reaction chamber, 2) new probes for detection of TB (IS6110 and IS1081) and rifampin resistance, and 3) optimized cartridge fluidics and polymer ase chain reaction (PCR) cycling. These changes improved the limit of detection from 130 colony-forming units (CFU)/mL to below 50 CFU/mL. The optimized assay detected 100% of smear-positive TB samples and 94% of smear-negative samples. Current sensitivity of the Xpert MTB/RIF assay is approximately 60% for smearnegative TB cases. There were also few rifampin-resistant false-positive results with the optimized assay. According to Alland, the new assay uses the same size (but different) cartridge, the time to assay results is the same, and the cost per sample is anticipated to be the same as that of the current assay in use. Field studies are underway, and results are eagerly awaited.

Early evaluations of the optimized Xpert MTB/RIF assay show sensitivity similar to that of a TB culture.

Transmission. Multidrug-resistant and extensively drug-resistant (XDR) TB pose threats to the progress being made in reducing TB burden and mortality. Shah and colleagues performed a cross-sectional study of 404 XDR TB isolates collected from KwaZulu-Natal, South Africa, from 2011 to 2014 in order to estimate the proportion of transmitted versus acquired drug resistance (Abstract 92). They performed IS6110-based restriction fragment length polymorphism genotyping and targeted sequencing of 9 resistance-conferring genes; for genotypic clusters with more than 20 participants, whole genome sequencing was performed. Cases were classified as ac-quired or transmitted based on a case definition and a genotypic analysis. Seventy-seven percent of participants were coinfected with HIV. Using the clinical case definition, 79% of cases were transmitted XDR TB and 21% were acquired XDR TB. With genotypic analysis, 87% of cases had an isolate that belonged to 1 of 16 clusters; only 13% had isolates that were unique. Forty-seven percent of cases were part of 1 large cluster, confirmed by whole genome sequencing. Other clusters ranged in size from 2 to 16 cases. These results suggest that the majority of diagnosed cases of XDR TB is attributable to transmission. Ongoing analysis of epidemiologic and geospatial data should provide information on transmission hot spots and lead to more effective approaches for interrupting person-to-person transmission of this difficult-to-treat disease.

Prior epidemiologic and modeling studies suggested that TB transmission is primarily driven by HIV-uninfected persons, in part because of the higher frequency of smear-negative TB among HIV-infected persons. New data from Malawi, using 1687 cultures (representing 72% of all culture-confirmed pulmonary TB cases), suggest that HIV-infected patients may be contributing to TB transmission more than originally thought (Abstract 816LB). Investigators examined patterns of TB transmission using genotypic methods and case histories. Glynn and colleagues estimated that 30.8% of HIV/TB-coinfected patients and 28.6% of HIV-uninfected TB-infected patients were the source of transmission for at least 1 additional case. This report underscores the point that dramatically reducing TB transmission will require prompt diagnosis and treatment of TB infection in both HIV-infected and -uninfected patients.

TB prevention. Danel and colleagues presented much awaited results from the Temprano study (Abstract 115LB), a randomized study (2x2 factorial design) comparing early antiretroviral therapy with antiretroviral therapy initiation according to WHO guidelines and isoniazid preventive therapy (IPT) with no IPT. Study participants were 2056 adults living in Ivory Coast who did not meet criteria for antiretroviral therapy initiation according to current WHO guidelines and had CD4+ counts less than 800 cells/μL. In terms of the outcome of the IPT randomization, IPT produced a 33% reduction in TB

cases. IPT was well tolerated and there was no evidence that IPT resulted in an increased number of TB cases with isoniazid resistance. This is the second randomized study showing that IPT adds an additional protective effect when used with antiretroviral therapy. As in the previous study from South Africa, this effect was seen in a population not selected for higher risk of TB, with confirmed latent TB. Now the challenge is implementing the evidence-based recommendations.

Six months of IPT is not the only effective TB prevention strategy. Short-course (3-month) preventive therapy with weekly isoniazid and rifapentine by directly observed therapy (DOT) is safe and effective for treating latent TB infection. However, DOT with this regimen may not be affordable in many settings. Belknap and colleagues conducted a randomized study to evaluate completion rates of this regimen using DOT, self-administered therapy (SAT), or self-administered therapy with weekly text reminders (eSAT) (Abstract 827LB). They prespecified a 15% noninferiority margin based on cost-effectiveness modeling in the United States.

The primary outcome was completion of more than 11 doses within 16 weeks. Of the 998 patients enrolled, 1% were HIV infected. Overall, treatment completion rates were 87.2% with DOT, 74.0% with SAT, and 76.4% with eSAT. Treatment completion rates in US participants were 85.4%, 77.9%, and 76.7%, respectively. SAT and eSAT were not found to be noninferior to DOT. However, SAT was found to be noninferior to DOT in a secondary analysis restricted to patients in the United States only. This study indicates that implementation of TB prevention therapy with 3 months of weekly isoniazid and rifapentine may require DOT in settings outside the United States.

Korenromp and colleagues provided more evidence that antiretroviral therapy reduces TB risk on a population level in an analysis using data from 41 countries (Abstract 832). Their analysis suggests that a 1% increase in antiretroviral therapy scale-up is associated with a 0.9% faster decline in TB deaths. Another interesting epidemiologic study from Durban, South Africa, addressed whether TB recurrence rates are reduced with antiretroviral therapy scale-up (Abstract 830). The case-defined TB recurrence was any case that occurred after treatment completion or any case in which there was history of a prior cured TB infection. Investigators compared time to first occurrence among those whose first visit occurred before (2000-2005) and after (2006-2012) the introduction of potent antiretroviral therapy. They found that TB recurrence risk decreased after antiretroviral therapy was scaled up in Durban and that the timing of these cases suggested that most were attributable to reinfection rather than recurrence. These studies support combined treatment with antiretroviral therapy and IPT as a maximal TB prevention strategy.

TB treatment and care. Boeree and Hoelscher presented the results of the open-label PanACEA MAMS-TB (Pan African Consortium for the Evaluation of Anti-tuberculosis Agents Multi-Arm-Multi-Stage-TB) study of new TB agents and high-dose rifampin (Abstract 95LB). This study aimed to identify regimen components that show promise for shortening of TB therapy. Investigators evaluated the investigational TB drug SQ109, moxifloxacin, and high-dose rifampin given with or as part of a backbone of isoniazid, pyrazinamide, and rifampin standard dosing. There were 4 intervention arms and 1 control arm in this study, which had a primary endpoint of time to culture conversion in liquid medium at 12 weeks. There was a planned interim analysis to discontinue study arms that showed a lack of benefit. The arm containing SQ109 and the arm containing SQ109 plus moxifloxacin were stopped prematurely during interim analysis owing to lack of efficacy. At the 12-week endpoint, the arms containing rifampin 20 mg/kg plus moxifloxacin or rifampin 35 mg/kg alone had faster times to culture conversion (55 days and 48 days, respectively, vs 62 days in the 4-drug control arm of isoniazid, rifampin, ethambutol, and pyrazinamide). Serious adverse event rates occurred in 6% of participants in the 2 intervention arms and in 5% of participants in the control arm. Hepatic adverse events were higher in the arm receiving the highest dose of rifampin than in the other 2 arms. These data suggest that higher levels of rifampin can achieve faster time to culture conversion in drug-sensitive TB infection, although hepatic toxicity rates were higher in the arm receiving the highest dose of rifampin. Clinical trials are ongoing to evaluate higher-dose rifampin as part of combination TB therapy.

High-dose rifampin shows promise for studies of shortened TB regimens.

Linkage to and retention in care remain an ongoing challenge for patients with HIV infection and for those coinfected with HIV and TB. Bassett and colleagues tested the hypothesis that the addition of a patient navigator to the care plan of patients newly diagnosed with HIV would improve outcomes (Abstract 93). They addressed this question among 4093 HIV-infected patients randomly assigned to receive a patient navigator or the standard of care. Navigators provided psychosocial support and text message and phone reminders. All patients were screened for TB at the time of diagnosis of HIV infection.

The primary composite endpoint of the study was antiretroviral therapy–eligible patients receiving at least 3 months of therapy and completion of TB therapy for those patients diagnosed with TB. Only 21% of patients in both arms reached the primary composite endpoint of the study. Further, 13% of the patients died during the 9 months of follow-up, with no difference between the arms. This report is an urgent call to action on the need to strengthen linkage to care, antiretroviral therapy initiation, retention in care, and coordinated TB services. The high mortality rates in this study are alarming.

Once patients are effectively linked to care, ensuring adherence to TB treatment remains an Achilles' heel of

effective TB control. In one of the most innovative TB presentations at CROI 2015, Browne and colleagues studied a new drug delivery system that permits real-time measurement of drug levels with an ingestible sensor and monitoring patch attached to a patient's torso (Abstract 828LB). Information from this device is transmitted to a paired mobile device that is uploaded to a secure network server, allowing health workers to confirm ingestions remotely (wireless observed technology [WOT]). Investigators integrated combination isoniazid and rifampin in an ingestible sensor with gel caps and then participants were randomly assigned to receive either standard pills or the gel caps with the sensor (WOT). Drug levels were bioequivalent between the 2 methods of delivery. Investigators also compared WOT with DOT in 280 simultaneous DOT ingestions. The WOT was well tolerated, with only 1 rash associated with the device. WOT detected more dosing than reported DOT. More data on the potential uses of this new technology to measure and monitor TB therapy are still to come.

Cryptococcal Meningitis

Sertraline is a selective serotonin reuptake inhibitor that shows activity against Cryptococcus neoformans in vitro and in murine models. To evaluate efficacy in humans, Rhein and colleagues conducted a phase IIb randomized study in Uganda, where frequency, morbidity, and mortality of cryptococcal meningitis remain high (Abstract 838). One hundred forty-four patients with cryptococcal meningitis were assigned to receive cryptococcal treatment (amphotericin B plus fluconazole) with sertraline at a daily dose ranging from 100 mg to 400 mg. The primary study endpoint was early fungicidal activity, defined as rate of cryptococcal clearance measured by serial cerebrospinal fluid cryptococcal cultures. Investigators found that in patients receiving sertraline, the organism was cleared 28% faster than in historical controls. Investigators also characterized the pharmacokinetics of sertraline: it reached steady state levels by 7 days, levels were in the predicted range of activity, and sertraline appeared synergistic with fluconazole in vitro. The clinical significance of these findings is undergoing evaluation in a larger randomized study.

Sertraline shows activity in vivo against cryptococcal meningitis.

Mortality rates for cryptococcal meningitis are high in sub-Saharan Africa, but few studies have compared mortality between patients developing cryptococcal meningitis before with after starting antiretroviral therapy. Rhein and colleagues measured 2-week mortality among 185 patients with cryptococcosis enrolled in a prospective cohort from August 2013 to August 2014 in Kampala, Uganda (Abstract 836). Forty percent were taking antiretroviral therapy at the time of the diagnosis of cryptococcal meningitis, and these patients had higher CD4+ cell counts and lower cryptococcal burden in cerebrospinal fluid than those not receiving antiretroviral therapy (4.0 $\log_{10}$ CFU/mL vs 4.8 $\log_{10}$ CFU/mL, respectively).

The 2-week mortality was significantly higher in those taking antiretroviral therapy for less than 14 days (54%) than in those taking antiretroviral therapy for 15 days to 4 months (16%; $P = .05$), for more than 4 months (12%; $P = .01$), or for those who were antiretroviral therapy naive (24%). This study shows that even patients who develop cryptococcal meningitis while taking antiretroviral therapy have a high mortality rate, and that mortality is extraordinarily high among those who present clinically soon after the initiation of treatment. These observations support screening for cryptococcal antigen before initiating antiretroviral therapy in patients who present for care with low CD4+ cell counts in places such as Uganda.

As mentioned above, mortality rates for cryptococcal meningitis are uniformly high in resource-limited settings. Ingle and colleagues sought to compile data from a 10-year period in North America and Europe from Collaboration of Observational HIV Epidemiological Research in Europe (COHERE), NA-ACCORD, and Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohorts, to assess mortality rates and analyze outcomes according to time between the initiation of antiretroviral therapy and the diagnosis of cryptococcal meningitis (Abstract 837). Among the 235 patients with available data, only 18% died within 6 months. The mortality rate was 11% within 6 months for 150 patients initiating antiretroviral therapy and did not differ between those who started therapy before or after diagnosis of cryptococcal meningitis (within 2 weeks). This study used dated observations but, nevertheless, shows that mortality rates for cryptococcal meningitis in developed-world settings are much lower than those reported from resource-limited settings.

Financial affiliations in the past 12 months: Dr Havlir has participated in research trials that have received provision of medicines from Gilead Sciences, Inc. Dr Currier has no relevant financial affiliations to disclose.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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- Developed by a panel of experts in the field.
- Provides practitioners with regularly updated, evidence-based, consensus recommendations for screening, treating, and managing patients with HCV.
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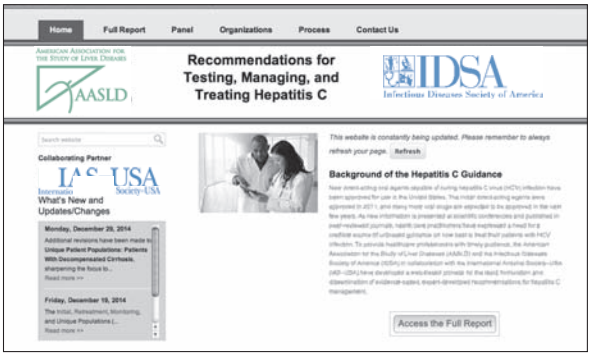
Recommendations for Testing, Managing, and Treating Hepatitis C

Recommendations for Testing, Managing, and Treating Hepatitis C is a website sponsored by the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) in collaboration with the International Antiviral Society–USA (IAS–USA) to provide the most current guidance for the treatment of hepatitis C virus (HCV).

Recently, several sections of the Guidance were extensively revised based on newly available therapies approved by the US Food and Drug Administration. Visit www.hcvguidelines.org to review the updates to sections on Initial Treatment of HCV Infection; Retreatment of Persons in Whom Prior Therapy Has Failed; Monitoring Patients Who Are Starting Hepatitis C Treatment, Are on Treatment, or Have Completed Therapy; and Unique Populations (Patients With HIV/HCV Coinfection, Patients With Decompensated Cirrhosis, Patients Who Develop Recurrent HCV Infection Post–Liver Transplantation, and Patients With Renal Impairement).



Available sections:

- HCV Testing and Linkage To Care
- When and in Whom to Initiate HCV Therapy
- Initial Treatment of HCV Infection
- Retreatment of Persons in Whom Prior Therapy Has Failed
- Monitoring Patients Who Are Starting Hepatitis C Treatment, Are on Treatment, or Have Completed Therapy
- Unique Patient Populations
  - Patients With HIV/HCV Coinfection
  - Patients With Decompensated Cirrhosis
  - Patients Who Develop Recurrent HCV Infection Post–Liver Transplantation
  - Patients With Renal Impairment
- Management of Acute HCV Infection

Review

CROI 2015: Highlights of Viral Hepatitis Therapy

Anne F. Luetkemeyer, MD; David L. Wyles, MD

High cure rates with all-oral regimens for patients with HIV/hepatitis C virus (HCV) coinfection were a highlight of the 2015 Conference on Retroviruses and Opportunistic Infections. Twelve weeks of sofosbuvir and daclatasvir led to sustained virologic response (SVR) rates of 96% in treatment-nave and 98% in treatment-experienced HCV genotype 1–infected patients. Twelve weeks of sofosbuvir plus ledipasvir had similar results, with SVR rates of 95% in treatment-naive and 96% in treatment-experienced patients. Patients with cirrhosis were included in both trials and attained SVR rates of 92% to 94%. Real-world performance of sofosbuvir and simeprevir resulted in SVR rates similar to those attained in clinical trials. Identifying HCV infection, linking patients to care, reducing barriers to drug access, and ensuring adherence will be key to realizing the enormous potential of high cure rates with interferon alfa–free therapies. Preventing reinfection after cure will be of particular importance in the HIV-infected population, which was highly impacted by reinfection rates of more than 20% during 5 years of follow-up in a meta-analysis.

Keywords: CROI 2015, hepatitis, HIV, HIV/HCV coinfection, direct-acting antivirals, DAAs, hepatitis C virus, HCV, sofosbuvir, ledipasvir, daclatasvir

Clinical Trials of Direct-Acting Antiviral Regimens in HIV/HCV Coinfection

Among the most anticipated hepatitis C virus (HCV) data from the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 22 to 26, were 2 late-breaking presentations that delivered the first sustained virologic response (SVR) with undetectable HCV RNA 12 weeks after therapy completion (SVR12) results from phase III studies of interferon alfa–free direct-acting antiviral (DAA) treatments in HIV/HCV-coinfected patients (Table 1). The ALLY-2 study evaluated sofosbuvir (a nonstructural protein [NS]5B polymerase inhibitor) and daclatasvir (an investigational NS5A antagonist) (Abstract 151LB), and the ION-4 study evaluated the fixed-dose combination of sofosbuvir plus ledipasvir (an NS5A antagonist) (Abstract 152LB). Both studies examined sofosbuvir plus an NS5A antagonist, evaluated 12 weeks of therapy in treatment-naive and -experienced patients (including those with cirrhosis), and omitted ribavirin from the regimen. Despite these similarities, there were a number of key differences. Given the broad genotypic coverage of daclatasvir, patients with HCV genotypes 1 to 6 were eligible for the ALLY-2 study (although only genotypes 1-4 were enrolled). Owing to predictable drug interactions and the ability to dose adjust daclatasvir, nearly all antiretroviral regimens were allowed in the study, including ritonavir-boosted (/r) HIV protease inhibitor (PI)-based regimens. Finally, ALLY-2 was the first trial to evaluate a shortened (8-week) therapeutic course for HCV treatment–naive, HIV/HCV-coinfected patients. The ION-4 study was more straightforward in design, evaluating a single fixed-dose combination in all patients for 12 weeks, and thus enrolled a larger number of patients and more patients with cirrhosis than did ALLY-2. Antiretroviral regimens were limited to tenofovir and emtricitabine in combination with either raltegravir, rilpivirine or efavirenz. Perhaps most importantly, sofosbuvir and ledipasvir are currently available to HIV/HCV-coinfected patients in the United States, but daclatasvir is not yet approved by the US Food and Drug Administration, although both regimens are approved for use in Europe.

Sofosbuvir and Daclatasvir

The ALLY-2 study enrolled 203 patients, including 101 treatment-naive patients who were treated for 12 weeks, 50 treatment-nave patients who were treated for 8 weeks, and 52 treatment-experienced patients who were treated for 12 weeks. The daclatasvir dose was adjusted to 90 mg for those taking efavirenz or nevirapine, to 30 mg for those taking a PI/r, and was kept at the standard 60 mg daily for all others. The majority of patients were men infected with HCV genotype 1a, 34% of patients were black, and 29% of treatment-experienced patients had cirrhosis.

The vast majority of patients had well-controlled HIV infection, with a median CD4+ count of greater than 500 cells/μL. The most common antiretroviral regimens included 2 nucleoside analogue reverse transcriptase inhibitors plus one of the following: raltegravir, efavirenz, atazanavir/r, or darunavir/r. Overall, treatment was safe and well tolerated, with good maintenance of HIV control. SVR12 rates for all patients with HCV genotype

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Table 1. Selected Phase III Clinical Trial Results for Patients Coinfected With HIV and HCV Genotype 1[a]

CROI 2015 Abstract (Study)	Regimen	No.	SVR12 Rate	Treatment Naive	Treatment Experienced	No Cirrhosis	Cirrhosis
151LB (ALLY-2)	Sofosbuvir and daclatasvir[b] for 12 weeks	127	97%	96% (80/83)	98% (43/44)	98%[c] (98/100)	91%[c] (20/22)
151LB (ALLY-2)	Sofosbuvir and daclatasvir[b] for 8 weeks	41	76%	76%	NA	78% (28/36)	60% (3/5)
152LB (ION-4)	Sofosbuvir and ledipasvir for 12 weeks	327	96%	95% (138/146)	97% (175/181)	96% (250/260)	94% (63/67)

Abbreviations: CROI, Conference on Retroviruses and Opportunistic Infections; HCV, hepatitis C virus; NA, not applicable; SVR12, sustained virologic response 12 weeks after cessation of therapy.

[a]See text for additional details. Subgroups calculated from data presented.

[b]Investigational drug in the United States.

[c]5 participants had indeterminate cirrhosis status at baseline; all achieved SVR12.

1 treated for 12 weeks were 96% for treatment-naive patients and 98% for treatment-experienced patients; response rates for all HCV genotypes were similar at 97% and 98%, respectively. Given these high SVR12 rates, no baseline factors clearly impacted response to 12 weeks of therapy, although relatively few patients with cirrhosis were present in each study arm (9 in the treatment-naive arm and 15 in the treatment-experienced arm).

Response rates with 12 weeks of therapy were 100% for genotype 2 and 100% for genotype 3. However, given the limited number of patients in the 12-week study arms, little can be said with certainty regarding the efficacy of treatment in these HIV/HCV-coinfected patients. Results from dedicated studies such as ALLY-3 are more reliable for predicting treatment response in individuals with HCV genotype 3 infection and still suggest limitations of 12 weeks of sofosbuvir and daclatasvir for treatment-experienced individuals with genotype 3 (SVR12 rate, 86%) and for those with genotype 3 and cirrhosis (SVR12 rate, 63%).[1]

In ALLY-2, the SVR12 rate was 76% with 8 weeks of treatment, substantially lower than the SVR12 rate of 97% to 98% seen with 12 weeks of treatment, and did not differ based on HCV genotype. This was surprising, given the high SVR rate (94%) attained in HCV-monoinfected patients with 8 weeks of a similar regimen of sofosbuvir plus ledipasvir.[2] Although ALLY-2 was not powered to detect differences in SVR by baseline factors after 8 weeks of treatment, patients taking darunavir/r were overrepresented in the 8-week arm and did have a numerically lower response rate (67%). Of note, drug-drug interaction data presented after the trial began indicate that darunavir/r does not increase levels of daclatasvir exposure to the same extent that atazanavir/r does.[3] As a result, current dosing guidelines recommend using 60 mg of daclatasvir for patients taking darunavir/r or lopinavir/r.

Sofosbuvir Plus Ledipasvir

The ION-4 study enrolled 335 patients in a single 12-week treatment arm of sofosbuvir plus ledipasvir. The majority of the patients were men (82%) with HCV genotype 1 infection (98%; patients with genotype 4 were also enrolled) and 34% were black. Slightly more than half of patients were treatment experienced (55%) and 20% had cirrhosis. Ninety-two percent of patients were taking raltegravir or rilpivirine, each with tenofovir and emtricitabine. HIV infection was well controlled, with a median CD4+ count of 628 cells/μL. Overall SVR12 rate was 96% (95% in treatment-naive patients and 97% in treatment-experienced patients). The presence of cirrhosis did not impact response rates. Curiously, all 10 patients who experienced viral relapse were black, including 8 who were taking efavirenz. This association of black race and viral relapse was statistically significant in a multivariate analysis, although no difference in sofosbuvir or ledipasvir exposure was found based on a population pharmacokinetic analysis. The explanation for the lower response rates remains unclear at this time.

Data were presented for the pharmacokinetic interactions of sofosbuvir plus ledipasvir and darunavir/r, atazanavir/r, or tenofovir in healthy volunteers (Abstract 82). The effect of antiretroviral drugs on sofosbuvir, sofosbuvir metabolite (GS-331007), and ledipasvir concentrations was not judged to be clinically significant.

However, sofosbuvir plus ledipasvir coadministered with darunavir/r raised tenofovir trough concentrations at end of dosing interval by 59% and area under the curve by 50%, and coadministration with atazanavir/r raised tenofovir trough concentrations by 47%. The mechanism was postulated to be persistent inhibition of efflux drug transporters. Ritonavir-boosted HIV PIs raise plasma tenofovir levels in the absence of sofosbuvir plus ledipasvir, thus this represents a further increase of tenofovir concentrations with the addition of sofosbuvir plus ledipasvir. The clinical impact of these elevated concentrations during 12 weeks of HCV therapy are unknown. Current prescribing information recommends avoiding coadministration of a PI/r and tenofovir with sofosbuvir plus ledipasvir, if feasible, or close monitoring of renal function if coadministration is necessary.

These data from the ALLY-2 and ION-4 trials represent the first phase III SVR data on contemporary DAA regimens for individuals with HIV/HCV coinfection. These data reinforce the notion that HIV/HCV-coinfected patients respond similarly to DAA

therapy as do HCV-monoinfected patients (with 12 weeks of therapy) and that sofosbuvir plus an NS5A antagonist is a remarkably potent and well-tolerated treatment option for this population. Drug interactions remain a limitation of therapy with sofosbuvir plus ledipasvir; however, the degree to which increased tenofovir concentrations during coadministration with sofosbuvir and ledipasvir will be seen in HIV/HCV-coinfected patients and, more importantly, whether this will be clinically significant remains unanswered. Clinical trial data for HIV/HCV-coinfected patients that address this potential interaction are eagerly awaited.

Hematologic Events With Paritaprevir/r, Ombitasvir, and Dasabuvir

The TURQUOISE-1 trial evaluated a regimen of paritaprevir/r (an HCV PI), ombitasvir (an NS5A inhibitor), and dasabuvir (a nonnucleoside NS5B polymerase inhibitor) given with weight-based ribavirin to HIV/HCV-coinfected patients for 12 weeks or 24 weeks.[4] In an analysis of grade 1 or 2 hematologic events, hemoglobin level declines occurred in 58% (18/31) of patients during 12 weeks of treatment and in 65% (21/32) of patients during 24 weeks of treatment, requiring ribavirin dose reductions for 4 and 2 patients, respectively (Abstract 691). All patients whose ribavirin doses were reduced attained an SVR.

Clinical Trials of DAA Regimens for HCV-Monoinfected Patients

Daclatasvir, Beclabuvir, and Asunaprevir

The UNITY-1 and UNITY-2 trials provided more data on the performance of a regimen of daclatasvir (an NS5A inhibitor), beclabuvir (an investigational nonnucleoside NS5B inhibitor), and asunaprevir (an investigational HCV PI). In the phase III UNITY-1 study, 12 weeks of this regimen given without ribavirin led to an SVR12 in 92% of HCV treatment-naive and 89% of treatment-experienced HCV-monoinfected patients with genotype 1 who did not have cirrhosis (Abstract 687). Comparing responses by HCV genotype, SVR12 rates were 8% higher in treatment-naive patients with genotype 1b than in those with genotype 1a, and 15% higher in treatment-experienced patients with genotype 1b than in those with genotype 1a. All confirmed virologic relapses were in patients with HCV genotype 1a. Baseline NS5A resistance did not negatively impact SVR in patients with HCV genotype 1b (100% [17/17] attained an SVR), whereas baseline resistance may have affected patients with HCV genotype 1a (74% [25/34] attained an SVR). Of the 25 patients who experienced virologic failure and had resistance test results available, 10 had resistance to all 3 drug classes and 14 had resistance to only NS5A or NS3.

Ribavirin may still be necessary for some subgroups treated with the investigational drugs daclatasvir, beclabuvir, and asunaprevir.

The UNITY-2 study examined the same 12-week regimen of daclatasvir, beclabuvir, and asunaprevir given with or without weight-based ribavirin to HCV genotype 1–monoinfected patients with cirrhosis (Abstract 688). In patients with HCV genotype 1a, the addition of ribavirin was associated with nonstatistically significant increases in SVR rates from 90% to 97% in treatment-naive patients and from 86% to 91% in treatment-experienced patients. Treatment-naive patients with HCV genotype 1b had a 100% SVR rate with or without ribavirin, and treatment-experienced patients with genotype 1b had SVR rates of 90% without ribavirin and 100% with ribavirin. Thus, in the presence of cirrhosis, there appears to be some benefit to the addition of ribavirin to patients with HCV genotype 1a and treatment-experienced patients with genotype 1b. It is unknown if ribavirin would improve SVR rates in HCV genotype 1a–infected patients without cirrhosis. As with the UNITY-1 study, baseline NS5A resistance did not appear to impact SVR. Of 13 treatment failures with resistance data available, 3 had resistance to all 3 drug classes and 8 had resistance to 1 or 2 drug classes. This regimen was generally well tolerated; as expected, the addition of ribavirin increased adverse effects, including anemia, fatigue, pruritus, and insomnia.

Collectively, these data demonstrate an overall high rate of SVR with the 12-week regimen of daclatasvir, beclabuvir, and asunaprevir and highlight that SVR rates are slightly decreased in patients with HCV genotype 1a and in those with prior treatment experience. Ribavirin may still be necessary for subgroups treated with this regimen, including patients with HCV genotype 1a and cirrhosis and treatment-experienced patients with genotype 1b and cirrhosis. Although virologic failures are uncommon, resistance to more than 1 drug class is common, and optimal retreatment strategies for these patients are needed.

Real-World Performance of DAA Regimens

Several studies examined response rates to DAA-based therapy outside the highly controlled settings of clinical trials. A German cohort examined outcomes of patients infected with HCV genotype 1 or 4 who were treated with sofosbuvir-based therapy. Of the 130 patients with SVR12 results available, approximately one-third were HIV infected. The 108 patients treated with sofosbuvir, peginterferon alfa, and ribavirin had an SVR12 rate of 85%, similar to the 90% response rate seen in a clinical trial.[4] The SVR12 rate for 15 patients treated with sofosbuvir and simeprevir was 87%, also similar to published clinical trial results.[5] HIV coinfection was not associated with decreased SVR rates; however, cirrhosis was associated with a 10% decrease in SVR12 rates ($P > .05$) (Abstract 646).

In another abstract that reported on real-world outcomes with sofosbuvir and simeprevir in 81 HCV genotype 1–infected patients, SVR12 rate was 77%

Table 2. Real-World Performance of Sofosbuvir and Simeprevir in HIV/HCV-Coinfected Patients by Pretreatment Fibrosis Stage and HCV Genotype[a]

	Cohort			SVR12			
CROI 2015 Abstract (Authors)	No.	Treatment Experienced	Metavir Fibrosis Stage F3 or F4	Genotype 1	Genotype 1a	Genotype 1b	Comments
644 (Marks et al)	15	100%	67% (F4 only)	93% (14/15)	--	--	All prior HCV PI treatment failures without detectable resistance
645 (Gilmore et al)	37	49%	78%	81% (30/37)	74% (17/23)	93% (13/14)	ITT analysis; 4/7 treatment failures were lost to follow-up (all 4 attained SVR4)
647 (Del Bello et al)	34	53%	56%	90% (26/29)	--	--	As treated
649 (Grant et al)	33	--	53% (F4 only)	95% (18/19)	92% (11/12)	100% (7/7)	As treated; 1 patient treated for 24 weeks

Abbreviations: CROI, Conference on Retroviruses and Opportunistic Infections; HCV, hepatitis C virus; ITT, intention to treat; PI, protease inhibitor; SVR4(12), sustained virologic response 4 (12) weeks after cessation of therapy.

[a]All patients treated for 12 weeks unless noted. Ribavirin used in some patients; unable to assess impact of ribavirin in these datasets.

in HIV/HCV-coinfected patients and 71% in HCV-monoinfected patients, and remained high (77%-78%) in the presence of cirrhosis. Half of the patients who did not attain an SVR12 were lost to follow-up and did not reflect confirmed virologic failures (Abstract 645). In several smaller cohorts of HIV/HCV-coinfected patients, many of whom were treatment experienced, treatment with sofosbuvir and simeprevir resulted in SVR12 rates of 93% to 95% (Abstracts 644 and 649) (Table 2). Of note, ribavirin was used in addition to simeprevir and sofosbuvir for some participants; however, studies were not powered to evaluate a difference in efficacy with and without ribavirin. These responses are similar to the SVR12 rates of 92% to 94% reported in the COSMOS (Combination of Simeprevir and Sofosbuvir in HCV Genotype 1–Infected Patients) trial.[6] Sofosbuvir-based HCV treatment was generally well tolerated, with the expected adverse effect of anemia when ribavirin was added to the regimen.

These studies continue to demonstrate that SVR12 rates with new oral HCV regimens outside of clinical trials are high and often approach those attained in clinical trials. Unlike what was seen in the interferon alfa era, current DAA regimens when used for HIV/HCV coinfection have generally not been associated with decreased cure rates or increased adverse events compared with HCV monoinfection. Loss to follow-up was an important contributor to failure to achieve an SVR. Thus, although current DAA regimens are much better tolerated than interferon alfa–containing regimens, there is a continued need for adherence support and posttreatment follow-up to ensure treatment completion and documentation of response.

Cost of DAA-Based Regimens

The high cost of HCV DAAs remains a central theme in discussions of new therapies and limits widespread use in those with HCV infection. Despite this high cost, new DAA regimens for HCV genotype 1 have generally been shown to be cost-effective when judged by the standard metric of an incremental cost-effectiveness ratio of approximately US $50,000 per quality-adjusted life-year.[7] Given the much-improved efficacy and tolerability profile of new DAAs, another key concept is analysis of the cost per cure when considering the total cost of treating persons with HCV infection.

Bichoupan and colleagues analyzed the cost per SVR of peginterferon alfa and ribavirin with telaprevir. In these initial studies, the cost per SVR was calculated to be approximately US $190,000.[8] In an updated analysis, Bichoupan presented data on 202 consecutive patients treated with sofosbuvir and simeprevir with or without ribavirin (173 patients had outcome data available) (Abstract 149). The patients treated in this series generally had a difficult-to-treat phenotype, were treatment experienced (70%, including 24% whose treatment with telaprevir or boceprevir had failed), and more than half had advanced fibrosis defined as a Fibrosis-4 (FIB-4) score of greater than 3.25. SVR rate for the cohort was 88% and mirrored that seen in clinical trials. The addition of ribavirin did not appear to impact SVR rate, although those who received ribavirin were enriched for prior failure and receipt of a PI.

In a multivariate analysis, prior telaprevir- and boceprevir-based treatment failure was associated with a sta-tistically significantly lower likelihood of achieving an SVR (odds ratio, 0.24; 95% confidence interval [0.09-0.63]; 76% SVR12 rate), perhaps

suggesting an impact of prior PI treatment failure and resistance to subsequent treatment with a cross-resistant component (simeprevir). Prior telaprevir- or boceprevir-based therapy has not been noted in studies to impact treatment with sofosbuvir plus an NS5A inhibitor or with paritaprevir/r, ombitasvir, and dasabuvir.[9,10] The cost per SVR was US $171,145, with drug costs making up 98% of this total. Still, this is lower than the cost per SVR seen with telaprevir-based therapy, reflecting the improved efficacy and tolerability of interferon alfa–free DAA regimens. Given the almost complete dependence of cost per SVR on drug costs, it is not surprising that in sensitivity analyses as the cost of drugs decreases so does the cost of SVR. With a 50% drug price discount (from wholesale prices) the cost per SVR was US $86,547.

Predictors of Adherence to DAA Regimens

Given the cost and increasingly shorter durations of DAA regimens, identifying predictors and barriers to adherence is key to treatment success with widespread implementation of DAAs in clinical practice. In an analysis of 2 clinical trials (SYNERGY and ERADICATE) evaluating treatment with ledipasvir plus sofosbuvir, adherence rate exceeded 90% and did not differ between HCV-monoinfected and HIV/HCV-coinfected participants, whether or not the latter were taking antiretroviral therapy (Abstract 692). Of note, there was a substantial decline in adherence over 12 weeks of therapy in all groups, emphasizing the need for adherence support and shorter regimens to optimize the success of DAA treatments. In 3 studies (SPARE, SYNERGY, and ERADICATE) evaluating sofosbuvir-based treatment, the presence of mental health disorders did not have a substantial negative impact on adherence or SVR rate (Abstract 694). However, these patients were enrolled in clinical trials and thus must have had relatively well-controlled psychiatric comorbidity.

Do HCV Viral Kinetics During DAA Therapy Matter?

The antiviral potency of current interferon alfa–free regimens has negated the predictive value of on-treatment HCV RNA kinetics in determining treatment course or outcome when evaluated in HCV monoinfection. Based on limited data, similar SVR rates are obtained with DAA therapies in individuals with HIV/HCV coinfection, yet to date no detailed data have been presented regarding on-treatment viral kinetics and outcomes in those with HIV/HCV coinfection taking regimens that contain more than 1 DAA drug. Several studies examined the predictive value of on-treatment HCV viral response in HIV/HCV coinfection.

An analysis of more than 2000 HCV-monoinfected and 60 HIV/HCV-coinfected patients treated with paritaprevir/r, ombitasvir, and dasabuvir with or without ribavirin for 12 weeks or 24 weeks compared the viral kinetics of HCV-infected patients with those of HIV/HCV-coinfected patients (Abstract 147). On-treatment viral kinetics were assessed by time to undetectable or detectable (but below the limit of quantification) HCV RNA and correlated with baseline characteristics and treatment outcomes (SVR12). Viral kinetics were similar between HCV-monoinfected and HCV/HIV-coinfected patients, with high SVR rates in both groups regardless of time to viral suppression. Of note, 100% of HIV/HCV-coinfected subjects had viral loads below the limit of quantification by week 4 of therapy. The only baseline factor associated with time to viral suppression was baseline viral load (higher viral load resulted in a longer time to viral suppression).

The predictive value of on-treatment HCV viral load monitoring was also evaluated in an analysis of 67 treatment-naive HCV-monoinfected or HIV/HCV-coinfected patients receiving ledipasvir plus sofosbuvir in the SYNERGY and ERADICATE studies (Abstract 689). A real-time polymerase chain reaction (RT-PCR) assay (Abbott, Abbott Park, IL) and an assay that measured HCV RNA in patients' blood (COBAS TaqMan HCV Test v1.0; Roche, Indianapolis, IL) were used. At week 4, 64% (43/67) of patients had detectable HCV RNA level by RT-PCR (22% [15/67] were quantifiable), and 27% (17/62) had detectable HCV RNA level in blood. At end of treatment, 18% (12/66) of patients had detectable but unquantifiable HCV RNA level by RT-PCR and none had detectable HCV RNA in blood. Only 1 patient experienced relapse after treatment. This analysis highlights the difference in detection thresholds between HCV viral load assays. Unlike the viral load responses seen in the peginterferon alfa era, low-level, detectable, on-treatment HCV viral loads at week 4 do not appear to predict DAA treatment failure, nor does HCV RNA detected below the limit of quantitation at end of treatment.

On-treatment viral kinetics do not determine treatment outcomes with newer, potent DAA regimens for HCV monoinfection or HIV/HCV coinfection.

Collectively, these data reinforce the notion that on-treatment viral kinetics are not useful for determining treatment outcomes with new, potent, interferon alfa–free DAA regimens for HCV monoinfection or HIV/HCV coinfection. This is a departure from viral load monitoring of patients taking interferon alfa–based therapies. However, a high on-treatment HCV viral load may indicate a lack of adherence. Thus, consistent with current guideline recommendations, viral load monitoring may be useful as a surrogate for adherence and may be prudent at select time points during HCV therapy.

Complications of HCV Infection

HCV/HIV coinfection has been associated with more-rapid progression of liver fibrosis and an elevated risk of complications such as renal insufficiency. However, it is unclear the extent to which effective antiretroviral therapy mitigates these complications.

In a North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) analysis of more than 34,000 HIV-infected adults with HCV or hepatitis B virus (HBV) coinfection, the overall rates of end-stage liver disease (ESLD) remained the same in the early (1996-2000), middle (2001-2005), and modern (2006-2010) antiretroviral treatment eras. ESLD rates remained markedly elevated, with a 6- and 7-fold increase in adjusted risk for HIV/HBV and HIV/HCV coinfection, respectively, compared with HIV monoinfection. Individuals with HIV/HCV/HBV coinfection were at highest risk for ESLD (Abstract 638).

To facilitate identifying those with elevated risk for hepatic complications, investigators from the EuroSIDA study reported a prognostic score that more accurately predicted liver-related death than fibrosis staging alone for HIV/HCV coinfection. This emphasizes the importance of clinical factors that contribute to hepatic morbidity and mortality, such as HBV coinfection, duration of HCV infection, and CD4+ cell count (Abstract 637). Female sex has been associated with slower fibrosis progression in HCV-monoinfected patients, but similar rates of fibrosis progression were seen in HIV-infected women and men in the ICONA (Italian Cohort of Antiretroviral-Naive Patients) study (Abstract 636).

Marijuana use has also been associated with liver fibrosis in HCV monoinfection in cross-sectional analyses. However, a large US cohort of HIV/HCV-coinfected women found no association between cannabis use and fibrosis progression. Of note, alcohol use was associated with marijuana use and independently associated with fibrosis progression (Abstract 639).

HCV-monoinfected veterans in the ERCHIVES (Electronically Retrieved Cohort of HCV-Infected Veterans) study database had a substantially increased overall risk of and accelerated time to chronic kidney disease (stage 3-5) after HCV antibody seroconversion than HCV-uninfected individuals, over a mean 5 years to 6 years of follow-up (Abstract 642). These data indicate that despite the availability of effective, less toxic antiretroviral therapy, HIV-infected patients remain at increased risk for accelerated fibrosis and liver-related complications.

Impact of Curative Therapy (SVR) on Clinical Outcomes

SVR has been associated with hepatic, nonhepatic, and mortality-related benefits. In an analysis of US veterans, SVR was associated with a substantially reduced risk of all-cause mortality in HIV/HCV-coinfected and HCV-monoinfected patients (Abstract 656). HCV-monoinfected patients with SVRs had a substantially reduced rate of hepatic decompensation, and there were no hepatic decompensations among HIV/HCV-coinfected patients who attained an SVR. SVR was also associated with substantial improvements in fibrosis measured by noninvasive markers in this study, and substantial reductions in liver stiffness measured by transient elastography and portal pressure measured by invasive hepatic venous pressure gradient in a Spanish cohort of HIV/HCV-coinfected patients (Abstract 657). In an Italian study, SVR was not associated with a substantial reduction in the incidence of diabetes, chronic kidney disease, cardiovascular disease, or death; however, the smaller sample size may have limited power for these endpoints (Abstract 655).

Impact of HCV Treatment on Immune Activation

Chronic immune activation is a common concern in HIV infection and may lead to long-term negative consequences. Chronic HCV infection may also lead to chronic immune activation, and with the advent of potent, interferon alfa–free treatments it is possible to monitor changes in immune activation during therapy and compare posttreatment profiles of patients.

C-X-C motif chemokine 10 (CXCL10; also known as gamma interferon–inducible protein 10 [IP-10]) is an interferon-stimulated chemokine that is consistently elevated in chronic HCV infection, and baseline levels of CXCL10 are predictive of responses to interferon alfa–based therapies. Meissner and colleagues evaluated dynamic changes of total CXCL10 during sofosbuvir-based, interferon alfa–free therapy for HCV infection (Abstract 681). They also measured concentrations of a truncated form of CXCL10, which is a negative regulator of immune activation, and measured activity of the enzyme dipeptidyl peptidase 4 (DPP-4), which catalyzes the cleavage of CXCL10. Consistent with previous studies,[11] they found that CXCL10 levels declined rapidly during HCV therapy in all 30 patients; truncated CXCL10 declined with similar kinetics. DPP-4 levels and activity also decreased but with slower kinetics and substantial decline was only seen by week 20. No difference in decline rates for any isoform was seen between those who achieved an SVR and those who relapsed. At baseline, patients with subsequent relapse did have substantially higher total (long-form plus truncated) and long-form CXCL10 levels.

Immune activation decreases in association with reductions with HCV viral load during effective therapy.

Wilson and colleagues presented data on immune activation in 10 patients with HIV/HCV genotype 1b coinfection with HIV suppression who were treated with an interferon alfa–free HCV regimen of asunaprevir and daclatasvir (Abstract 683). The key finding was that immune activation, measured by expression of HLA-DR + CD38+ in CD4+ and CD8+ T cells, decreased substantially from baseline to day 140 of therapy. CD4+ cell activation decreased by 21% (P = .023) from baseline, and CD8+ cell activation decreased by 12% (P = .018). SVR data and follow-up samples were not presented to assess for increasing immune activation in those patients who relapsed after therapy. Comparisons were also not made to HIV-suppressed patients who were not coinfected with HCV.

These abstracts collectively demonstrate that immune activation decreases in association with reductions in HCV viral load during effective therapy. At this point, studies have been insufficient to determine whether assessments of immune activation before or during therapy may hold some promise for predicting treatment responses to DAA drugs. Treating and curing HCV would certainly seem to be another component to reducing overall immune activation in HIV/HCV-coinfected patients.

Timing of HCV Treatment: Impact of Deferring HCV Therapy

The high cost of HCV therapy combined with the reality of limited medical resources have resulted in the imposition of treatment restrictions based on fibrosis stage in many resource-rich settings. Based on the recognition that those with advanced fibrosis or cirrhosis are most likely to suffer near-term clinical consequences from their HCV-related liver disease, treatment restrictions for those with advanced liver disease have been widely enacted by payers.

Zahnd and colleagues presented modeling data based on fibrosis progression and the clinical endpoints of decompensated liver disease, hepatocellular carcinoma (HCC), and liver-related mortality (Abstract 150). It was assumed that SVR decreased fibrosis progression and development of decompensated liver disease by 10-fold each. HCC risk was estimated to decrease by 2.6-fold following SVR. In the base case scenario, progression to the clinical endpoints over time was evaluated in the Swiss HIV Cohort Study population, assuming a 60% treatment uptake rate and 40% cure rate with peginterferon alfa and ribavirin in HIV/HCV-coinfected patients. These estimates seem optimistic, particularly for treatment uptake with interferon alfa–based therapies. Based on these assumptions, the model predicted a 25% rate of lifetime mortality from HCV-related liver disease.

Transitioning to DAA therapy, the investigators adjusted the uptake rate to 100% and the cure rate to 90%. They then modeled the impact of treatment at various time points and stages—from within 1 month or 1 year of HCV infection to having stage 2 to 4 liver fibrosis. In the model, treating within 1 month or 1 year of infection did not impact outcomes differently, with each resulting in a dramatic reduction in liver-related deaths (to <3%). Modest increases in clinical, liver-related endpoints were seen if therapy was delayed until Metavir fibrosis stage F2; however, the largest increases were seen when therapy was delayed until Metavir fibrosis stage F3 or F4 (rates of HCC and liver-related death in those with Metavir stage F4 were comparable to or higher than those seen with interferon alfa–based treatment). The rates of lifetime liver-related mortality were 25%, 10%, and 5% for treating at Metavir stages F4, F3, and F2, respectively. It was sobering that most deaths in those who had Metavir stage F3 or F4 at the time of treatment occurred after completion of HCV therapy.

It seems obvious that intervention in a disease process at an earlier time point would produce benefits relative to waiting for an advanced disease state to develop, whether it be cardiovascular disease, HIV infection, or malignancies. Following HCV cure, liver disease progression may not immediately halt. Although dramatically diminished, the risk of HCC persists, and other hepatic insults (eg, alcohol use or nonalcoholic steatohepatitis) may continue to push patients to morbid clinical endpoints even after they have been successfully treated. Thus, treating HCV-induced liver fibrosis at an earlier stage would be associated with clinical benefits. However, clinical trial data to support this are lacking and will be difficult and expensive to obtain. In this regard, modeling data can be a useful starting point to attempt to quantitate the impact of HCV cure at various disease stages, despite obvious limitations and inherent assumptions.

HCV Resistance

The clinical relevance of resistance to HCV DAA drugs, owing to preexisting polymorphisms or selection after exposure to an antiviral drug, remains unclear. Much of this undoubtedly relates to the ongoing rapid evolution of therapies themselves and the high efficacy of current DAA therapies that results in few treatment failures in studies. It has been well described that the resistance barrier to DAA drugs may differ between HCV genotype subtypes 1a and 1b.[12]

Cook and colleagues presented a comprehensive overview of NS5A resistance in 109 HCV genotype 1 virus (1a, 71; 1b, 38) samples analyzed by ultra-deep sequencing of virus, with resistant variant detection rates compared with population sequencing (Abstract 696). Variants at NS5A positions 28, 30, 31, 32, 58, and 93 were considered. Frequency and susceptibility of commonly detected resistance-associated variants (RAVs) was assessed in a phenotypic assay using HCV genotype 1a and 1b replicons. Consistent with prior studies, NS5A RAVs were detected more frequently in samples of HCV genotype 1a than in genotype 1b, with 38% (27/71) of genotype 1a viruses harboring NS5A RAVs compared with 21% (8/38) of genotype 1b viruses. RAVs at position 28 were most frequent in HCV genotype 1a viruses, and position 93 RAVs were most frequently seen in genotype 1b.

The resistance barrier to DAA drugs may differ between HCV genotype subtypes 1a and 1b.

In addition to an impact on prevalence, the phenotypic impact on susceptibility to NS5A inhibitors also tends to be larger in HCV genotype 1a than in 1b. This observation was borne out in the phenotypic assay results presented, in which the average fold-change in 50% inhibitory concentration of an NS5A inhibitor was approximately 10-fold higher for RAVs in HCV genotype 1a backbone (average fold increase of >150 in genotype

Table 3. HCV Direct-Acting Antiviral Drug Classes and US FDA Approval Status

HCV Direct-Acting Antiviral Class	Drug (year/status of US FDA approval)
NS3 Protease inhibitors	Simeprevir (2013) Paritaprevir/ritonavir (2014) Asunaprevir (not approved)
NS5B Nucleos(t)ide polymerase inhibitor	Sofosbuvir (2013)
NS5B Nonnucleoside polymerase inhibitors	Dasabuvir (2014) Beclabuvir (not approved)
NS5A Inhibitors	Ledipasvir (2014) Ombitasvir (2014) Daclatasvir (not approved)

Abbreviations: HCV, hepatitis C virus; NS, nonstructural protein; US FDA, US Food and Drug Administration.

1a vs an average fold increase of <20 in genotype 1b). In clinical practice, only population sequencing results will be available; however, in this study, population sequencing only detected RAVs in 31% of the samples in which RAVs were detected through ultradeep sequencing. A clinically significant threshold for RAV prevalence in quasispecies has not yet been determined.

As preferred HCV regimens evolve, the necessity of determining the presence of a baseline Q80K RAV, which is associated with decreased response to simeprevir, is likely to continue to wane. However, accurate determination of HCV genotype subtype remains crucial to determining the components (whether to include ribavirin) and durations of newer interferon alfa–free treatments.

Joy and colleagues presented data on NS3 deep sequencing of 376 clinical samples of HCV genotype 1 in British Columbia, Canada, to determine Q80K-related resistance status (Abstract 697). The most interesting results from this study were not the data on Q80K prevalence—this was much higher in HCV genotype 1a than in 1b, as expected—but rather the striking number of instances of incorrect subtyping or of inability to determine subtype using a line probe genotype assay, which was resolved using deep sequencing. Ninety-six percent (52/54) of HCV genotype 1 samples whose subtype had not been determined were classified as genotype 1a using NS3 deep sequencing, as were 44% (28/91) of samples previously categorized (using the line probe assay) as genotype 1b. Although using deep sequencing to determine genotype subtype is impractical for patient care, these results suggest that clinicians who receive a genotype testing result indicating that HCV genotype 1 cannot be subtyped should treat these patients as genotype 1a. This is already a conservative approach, as treatment regimens for HCV genotype 1a are longer in duration or include ribavirin in some DAA regimens in select situations. Of course, it is unclear whether this finding is widely applicable or a unique phenomenon in HCV genotype 1 viruses circulating in British Columbia. The 11% prevalence rate of the Q80K mutation in samples of HCV genotype 1b determined using the line probe assay is higher than that seen in other studies examining genotype 1b isolates using the same assay, suggesting the assay may be particularly prone to incorrect subtyping for HCV genotype 1 viruses circulating in British Columbia or that perhaps a higher prevalence of genotype 1b isolates in British Columbia do harbor the Q80K mutation.

Screening for HCV

Recommendations for birth cohort screening for HCV have been in place since 2013.[13] These initial recommendations were based on the estimate that roughly 75% of HCV-infected individuals in the United States were born between 1945 and 1965. Using data from a large, national clinical laboratory, Klevens and colleagues presented data on the use of FIB-4 score to quantify the frequency of HCV-related advanced liver disease across different age-based cohorts (Abstract 145).Using Using FIB-4 score as a surrogate for liver disease staging (advanced fibrosis and cirrhosis categorized as FIB-4 scores of 2.0-3.7 and >3.7, respectively), estimates of the proportions of persons with advanced fibrosis or cirrhosis were presented for different age groups. As expected, the proportion of persons with advanced fibrosis or cirrhosis increased based on age, ranging from 11% in those born after 1965 to almost 75% in those born before 1945. Within the birth cohort of those born between 1945 and 1965, 47% of persons were estimated to have advanced fibrosis or cirrhosis; perhaps more importantly, persons in this birth cohort account for 81% of cases of advanced fibrosis or cirrhosis in the United States.

HCV-infected individuals born between 1945 and 1965 account for 81% of cases of advanced fibrosis in the United States.

Available data suggest that uptake of birth cohort HCV screening has been limited among primary care practitioners. In 2 abstracts presented by Brodsky and colleagues (Abstract 658) and Tzarnas and colleagues (Abstract 668), respectively, investigators first assessed practitioner knowledge of current HCV therapeutics and screening recommendations and then evaluated the impact of educational and electronic medical record (EMR)-based prompts to improve screening approaches and coverage. In a practice-level survey of 7 primary care practices, including 57 primary care practitioners and 42 clinic support staff members, investigators found that knowledge of current HCV treatment efficacy and durations was poor (Abstract 658). Only 40% of practitioners were aware that cure rates of greater than 70% were attainable for HCV infection, and slightly more than 60% of practitioners were able to identify recommended treatment duration ranges. HCV screening in line with Centers for Disease Control and Prevention (CDC) and US Preventive Services Task

Force (USPSTF) recommendations[13,14] was poor despite a reasonable level of awareness of testing guidelines (68%). Modest improvements in frequency of HCV screening with education and EMR prompts based on birth cohort were seen over time, although no site increased testing by more than 50% of the targeted population within 2 months of implementing an EMR prompt system.

In the second abstract, a more detailed description and analysis of the impact of an educational and EMR prompt–based approach were presented. Before education and EMR prompting, only 7% of eligible persons were screened. Institution of the EMR prompt program increased this rate to 18% to 20%, suggesting some effectiveness but highlighting the fact that other approaches to increase HCV screening in primary care are needed. Persistent perceptions of a lack of efficacy of HCV therapy or concerns that it will not be covered for many patients (owing to payer limitations) may contribute to the lack of widespread HCV screening. It is encouraging that modification of HCV screening orders in EMRs did have a dramatic effect on facilitating appropriate testing. Identification of individuals with HCV infection who are already linked to care is key to realizing the benefits of improvements in HCV therapy. Clearly, additional education of practitioners and clinic staff on HCV testing guidelines and advancements in therapy are needed.

Other data presented at CROI 2015 also support the notion that widespread adoption of CDC/USPSTF HCV screening guidelines has not yet occurred in clinical practice. In a CDC-funded project in Washington, DC, only 31% of more than 4000 eligible persons were screened for HCV during a 2-year study (Abstract 666). Of concern, a higher than expected HCV seroprevalence rate of 7.5% was found, including 13% in black men within the cohort. In a complementary emergency department (ED)-based study conducted in Baltimore, Maryland, all patients who had blood drawn in the ED were tested for HCV antibody (samples were de-identified) to assess the impact of CDC recommendations for HCV testing (Abstract 667). Over 8 weeks 4713 ED patients were tested for HCV; seroprevalence rate was 13.8%, with approximately two-thirds of those patients having previously diagnosed HCV infection. Although adding CDC recommendations for birth cohort screening to risk-based screening increased HCV detection 2-fold, up to 25% of unknown HCV infections would still have been missed. These findings suggest that routine screening of all persons presenting to the ED may be of value in settings with higher HCV prevalence rates, such as most urban EDs.

HCV Prevalence and Linkage to Care

Two studies highlighted the substantial prevalence of HCV infection and the many practical hurdles that remain for integrating HCV therapy into the care of poorer, generally minority populations in urban settings. In a longitudinal study of HIV-seropositive persons in care at 1 of 13 centers in Washington, DC, a high HCV prevalence rate of 13.3% was documented, including a remarkable incidence of 1.56 HCV infections per 100 person-years (Abstract 660). Following guidance from the American Association for the Study of Liver Diseases/Infectious Diseases Society of America/International Antiviral Society–USA (AASLD/IDSA/IAS–USA) Recommendations for Testing, Managing, and Treating Hepatitis C on which patients should be prioritized for HCV therapy (http://www.hcvguidelines.org),[15] more than 70% of patients in the study had factors placing them at high priority for HCV therapy (22% had evidence of advanced fibrosis or cirrhosis).

Data from community-based HCV testing and linkage-to-care programs in San Diego, California, identified a similarly high rate of HCV prevalence in at-risk, vulnerable populations and showed a drop-off in treatment rates associated with lack of insurance and medication coverage (Abstract 661). Of 1634 persons tested, 18% were HCV seropositive. In a traditional assessment of linkage to care and HCV treatment, 94% of persons had an HCV RNA performed (likely reflecting point-of-care HCV antibody testing with reflex phlebotomy) and 71% had a positive HCV RNA result. Despite this, only 45% of individuals were linked to care and only 7% of those initiated HCV therapy. Failure to link individuals to care and lack of insurance coverage for HCV therapy were major barriers to treatment.

Acute HCV Infection

HIV-infected men who have sex with men (MSM) are increasingly recognized as being at risk for HCV infection and for reinfection after HCV treatment. In an important reminder of this risk, an observational German study of 212 HIV-infected MSM with acute HCV infection reported that 14.6% of participants were subsequently reinfected with HCV after cure or spontaneous viral clearance. Injection drug use, with ever injected drugs reported at 36%, and sexual exposure were risk factors for HCV transmission (Abstract 671). Spontaneous viral clearance occurred in 10% of participants. The IPERGAY study of preexposure prophylaxis (PrEP) reported 4 acute HCV infections, a reminder that men at risk for HIV infection should be counseled on the risk of sexually acquired HCV as well (Abstract 23LB). In addition, prescribers of PrEP should be vigilant about the possibility of acute HCV infection as well as other sexually transmitted infections.

MSM are increasingly recognized as being at risk for HCV infection and for reinfection after HCV treatment.

In a cohort of men newly diagnosed with HIV infection in Los Angeles, California, more than half of whom were recently infected, prevalence of HCV coinfection was low (1.6%) at the time of HIV diagnoses (Abstract 672). This suggests that HCV infection may be occurring after HIV infection in some MSM, highlighting the opportunities for targeted counseling and HCV prevention strategies for MSM newly diagnosed with HIV infection. In

Amsterdam, a case-control study of acute HCV infection in HIV-infected patients identified sexual risk behaviors for HCV acquisition: receptive unprotected anal intercourse, sharing of sex toys, a recent ulcerative sexually transmitted infection, and unprotected fisting. Nonsexual risk factors were injection drug use, sharing of noninjection drug equipment (ie, straws), and lower CD4+ cell count (Abstract 674). Modeling by the Swiss HIV Cohort Study predicts that even with widespread implementation of effective HCV therapy, stabilization or reduction in high-risk sexual behaviors will be necessary in order to curb the HCV epidemic (Abstract 675).

With regard to treatment of acute HCV infection, 12 weeks of boceprevir with peginterferon alfa and ribavirin resulted in an SVR12 rate of 76% (26/34) in HIV-infected patients with acute HCV infection (treatment initiated within 26 weeks of infection), indicating that treatment duration with an HCV PI and peginterferon alfa can be cut in half and still maintain the same response rates (Abstract 669). Whether shortened courses of all-oral DAA therapy can be used to effectively treat acute HCV infection is unknown but is under investigation.

HCV Recurrence After HCV Therapy

In a meta-analysis of 49 studies and more than 8000 participants, risk of HCV infection recurrence varied by risk categorization and HIV serostatus. Participants categorized as having high risk included injection drug users and those who were incarcerated; categorization as low risk excluded those with HIV coinfection, injection drug users, and those who were incarcerated. Data on sexual risk for HCV reinfection were not presented. The 5-year risk of HCV infection recurrence was 1.14% among low-risk HCV-monoinfected patients, 13.2% among high-risk HCV-monoinfected patients, and rose to 21.7% among HIV/HCV-coinfected patients. Given the association with elevated risk, recurrent HCV infection was largely attributed to reinfection rather than late relapse (Abstract 654). In 8 National Institutes of Health studies of sofosbuvir and ribavirin or sofosbuvir plus ledipasvir in HCV-monoinfected and HIV/HCV-coinfected patients, 100% of patients who attained an initial SVR12 remained free of HCV infection during an average 35 weeks of follow-up, with the exception of one phylogenetically confirmed reinfection (Abstract 653). These data reinforce SVR12 as a biomarker for long-term HCV cure and are a sobering reminder that patients cured of HCV infection remain at risk for re-infection, with HIV/HCV-coinfected patients having a markedly higher risk.

Other Viral Hepatitides

HBV Vaccination Strategy in HIV Infection

Attaining protective antibody titers with HBV vaccination is an ongoing challenge for many HIV/HBV-coinfected patients. Among HIV-infected patients with CD4+ counts greater than 200 cells/μL who did not respond to initial HBV vaccinations, revaccination with a double dose of HBV vaccine did not significantly increase hepatitis B surface antibody (HBsAb) response compared with a standard dose (67% vs 74%, respectively; $P = .2$) in a randomized clinical trial (Abstract 701). However, double-dose vaccination was associated with high HBsAb titers (>100 IU/mL) and with a protective response 72 weeks after vaccination. CD4+ cell count was not associated with increased response to vaccination. Double-dose HBV vaccination has been associated with higher seroconversion rates in some studies,[16] particularly with CD4+ counts greater than 350 cells/μL.[17] Although it did not demonstrate an increase in overall seroconversion rates, this study indicates there may be a benefit of longer duration or higher titers in those who respond to the double-dose vaccine strategy.

Hepatitis Delta Virus and Hepatitis E Virus

Hepatitis delta virus (HDV) and hepatitis E virus (HEV) are relatively uncommon viral coinfections of HIV; however, both can cause serious hepatic disease and may go unrecognized. HDV viremia was present in 2 of 138 HIV/HBV-coinfected patients in Ohio (Abstract 707). Although the overall rate of HDV prevalence was low among these patients, HDV was not suspected in the 2 patients who had it, suggesting that HDV infection may go undiagnosed in some US populations. Identification of HDV is clinically important, as it is associated with more-rapid disease progression and may respond to treatment. In a double-blind phase IIa study, use of the investigational oral prenylation inhibitor lonafarnib caused a substantial decline in HDV RNA levels during 28 days of therapy in HDV-infected patients without HIV coinfection (Abstract 708LB). Lonafarnib may be a promising alternative to interferon alfa, which is poorly tolerated and has suboptimal efficacy for HDV treatment.

Investigating the incidence and clinical impact of HEV infection among HIV-infected patients, a prospective Spanish study found that 5% of patients developed HEV infection during a median 12 months of follow-up. HEV seroconversion was associated with clinical symptoms in 58% of patients, including fever and abdominal pain. Compared with HIV-infected patients without HEV infection, HIV/HEV coinfection was associated with a significant increase in transient HIV viremia (35.0% vs 3.8%, respectively; $P < .001$) and frank hepatic decompensation in patients with cirrhosis (33.3% vs 2.1%, respectively; $P < .001$) (Abstract 709).

Financial affiliations in the past 12 months: Dr Luetkemeyer has received grants awarded to her institution from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Pfizer, Inc. Dr Wyles has received grants awarded to his institution from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, and Merck & Co, Inc, and serves on an advisory board for Bristol-Myers Squibb.

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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