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.

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

Additional References

1. World Health Organization (WHO). WHO issues new HIV recommendations calling for earlier treatment. http://www.who.int/mediacentre/news/releases/2013/new_hiv_recommendations_20130630/en/. Accessed on April 8, 2015.
2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf. Accessed on April 8, 2015.
3. Centers for Disease Control and Prevention (CDC). Vital signs: HIV prevention through care and treatment--United States. *MMWR Morb Mortal Wkly Rep*. 2011;60(47):1618-1623.
4. Joint United Nations Programme on HIV/AIDS. 90-90-90: An ambitious treatment target to help end the AIDS epidemic. http://www.unaids.org/en/resources/documents/2014/90-90-90. Accessed on April 9, 2015.
5. World Health Organization. *WHO Recommendations on the Diagnosis of HIV Infection in Infants and Children*. Geneva, Switzerland: World Health Organization, 2010.
6. Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed on April 15, 2015.
7. Wensing AM, Calvez V, Gunthard HF, et al. 2014 update of the drug resistance mutations in HIV-1. *Top Antivir Med.* 2014;22(3):642-650.
8. Johnson VA, Calvez V, Gunthard HF, et al. Update of the Drug Resistance Mutations in HIV-1: March 2013. *Top Antivir Med.* 2013;21(1):6-14.
9. Hazuda DJ, Young SD, Guare JP, et al. Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science.* 2004;305(5683):528-532.

Top Antivir Med. 2015;23(1):28-45.