Advances in Antiretroviral Therapy

Susan Olender, MD, Timothy J. Wilkin, MD, MPH, Barbara S. Taylor, MD, MS, and Scott M. Hammer, MD

The 19th Conference on Retroviruses and Opportunistic Infections (CROI) highlighted new information and provided in-depth discussion on advances in antiretroviral therapy (ART). Data regarding investigational drugs, including integrase strand transfer inhibitors (InSTIs) and zinc-finger nucleases disrupting CC chemokine receptor 5 (CCR5), were presented. Treatment trials in treatment-naive and treatment-experienced patients added to the knowledge base of which antiretroviral agents to initiate and when. Data from trials and observational cohorts suggested that, for patients on successful ART in resource-rich settings, mortality from non-HIV-related diseases may surpass that from HIV-related diseases, and overall lifespan may be nearing that of people without HIV infection. In resource-limited settings (RLS), prevention of mother-to-child transmission (PMTCT) and ART scale-up remained priorities. New data on antiretroviral resistance in RLS and on the implications of low-frequency mutations were presented.

New Antiretroviral Agents

GS-7340

GS-7340 is a prodrug of tenofovir and results in the same active compound as tenofovir disoproxil fumarate: tenofovir-diphosphate. At the 2012 Conference on Retroviruses and Opportunistic Infections (CROI), Ruane and colleagues presented results from a partially blinded, controlled, dose-escalation study comparing GS-7340 (8 mg, 25 mg, and 40 mg once daily), tenofovir 300 mg once daily, and placebo given as monotherapy for 10 days (Abstract 103). This trial enrolled 38 HIV-infected participants, of whom 97% were men; mean HIV-1 RNA level was 4.5 $\log_{10}$ copies/mL; mean CD4+ count, 478 cells/μL. As expected, plasma tenofovir exposures were 80% to 97% lower in the GS-7340 arms than in the tenofovir arm, and the intracellular tenofovir-diphosphate levels were higher in the GS-7340 arms than in the tenofovir arm. The time-averaged change in plasma HIV-1 RNA levels through 10 days was greater in the GS-7340 25 mg and 40 mg arms (-0.94 $\log_{10}$ copies/mL and -1.13 $\log_{10}$ copies/mL, respectively) than in the tenofovir arm (-0.48 $\log_{10}$ copies/mL, $P = .01$ and $P = .001$, respectively). The investigators assert that GS-7340 has the potential to be more efficacious with less systemic toxicity than tenofovir. This will be investigated further in phase II trials.

CCR5 Disruption by Zinc-Finger Nucleases

Tebas and colleagues presented data from 2 phase I, single-arm, single-dose clinical trials of zinc-finger nuclease–modified autologous CD4+ T cells (SB-728-T) (Abstract 155). This strategy uses apheresis to collect large numbers of CD4+ cells, which are then exposed ex vivo to zinc-finger nucleases that target and disrupt the CC chemokine receptor 5 (CCR5). The genetically modified CD4+ cells are expanded, cryopreserved, and infused back into the participant. The studies enrolled 6 immune responders with CD4+ counts at least 450 cells/μL (median count 974 cells/μL) and 15 immune non-responders with CD4+ counts below 450 cells/μL (median count 357 cells/μL). All participants had virologic suppression on combination antiretroviral therapy (ART).

The infusions were generally well tolerated. One serious adverse event, a transfusion reaction of arthritis, was reported. In the immune-responder group, the mean CD4+ count increased at day 7 postinfusion by 1533 cells/μL, including 83 cells/μL of genetically modified CD4+ cells. Increases of 820 CD4+ cells/μL and 19 genetically modified CD4+ cells/μL were observed after infusion in the immune–non-responder group. Genetically modified CD4+ cells were detected at 1 year of follow-up in approximately 2% of circulating CD4+ cells. In participants undergoing a treatment interruption, plasma HIV-1 RNA levels rebounded. The frequency of genetically modified CD4+ cells correlated with control of viremia during the treatment interruption. Future studies will focus on increasing the efficiency of engrafting modified CD4+ cells to enhance virologic control.

Inhibitors of Integrase Complexes

Current integrase strand transfer inhibitors (InSTIs) target enzymatic activity of HIV integrase. Gros and colleagues described a short 11-mer cyclic peptide that binds integrase and causes dissociation of integrase–DNA, integrase–lens epithelium-derived growth factor (LEDGF), and reverse transcriptase (RT)–integrase complexes (Abstract 576). The compound exhibited low nanomolar activity against a broad range of HIV isolates, and the investigators were unable to generate resistance to the peptide after a 12-month evaluation.

Dr Olender is Instructor of Medicine at Columbia University Medical Center. Dr Wilkin is Associate Professor of Medicine at the Weill Cornell Medical College. Dr Taylor is Assistant Professor of Infectious Diseases at the University of Texas Health Science Center San Antonio. Dr Hammer is Harold C. Neu Professor of Medicine at the Columbia University College of Physicians and Surgeons and Chief of the Division of Infectious Diseases at Columbia University Medical Center.

Nanoformulations of Atazanavir/Ritonavir

Gendelmen and colleagues presented data on the pharmacokinetics on nanoparticle formulations of ritonavir-boosted (/r) atazanavir in a mouse model (Abstract 582). They tested increasing doses of atazanavir/r and were able to achieve atazanavir concentrations of 287 ng/mL with weekly injections. The nanoparticles resulted in partial virologic suppression (HIV RNA 2 $\log_{10}$ copies/mL) in a humanized mouse model of HIV infection.

Clinical Trials of Antiretroviral Therapy of Treatment-Naive Individuals

Elvitegravir/Cobicistat/Tenofovir/Emtricitabine

Sax and colleagues presented data from a phase III, randomized, double-blind, placebo-controlled trial of a fixed-dose combination of elvitegravir/cobicistat/tenofovir/emtricitabine (elvitegravir/cobicistat arm) versus fixed-dose efavirenz/tenofovir/emtricitabine (efavirenz arm) (Abstract 101). Elvitegravir is an investigational InSTI, and cobicistat is an investigational pharmacoenhancer. The trial enrolled antiretroviral-naive adults with plasma HIV-1 RNA levels greater than 5,000 copies/mL, calculated creatinine clearance greater than 70 mL/min, and no resistance to efavirenz/tenofovir/emtricitabine. Seven hundred participants (89% male; 37% nonwhite) were randomly assigned to the 2 treatment groups. The mean CD4+ counts were 382 cells/μL and 391 cells/μL in the 2 arms, respectively, and 33% of participants had plasma HIV-1 RNA levels greater than 100,000 copies/mL.

Viral suppression by the US Food and Drug Administration (FDA) snapshot algorithm at week 48 was 88% and 84% (difference, 3.6%; 95% confidence interval [CI], -1.6%-8.8%) in the elvitegravir/cobicistat and efavirenz arms respectively. The elvitegravir/cobicistat arm was found to be noninferior to the efavirenz arm. The mean CD4+ count increases were 239 cells/μL and 206 cells/μL (P = .009), respectively. Drug discontinuations due to adverse events occurred in 4% and 5% of the treatment groups, respectively. Nausea was more common in the elvitegravir/cobicistat arm (21% vs 14%); central nervous system effects (CNS) and rash were more common in the efavirenz group. Calculated creatinine clearance decreased by 14.3 mL/min after 2 weeks in the elvitegravir/cobicistat group due to the known inhibition of tubular secretions of creatinine by cobicistat. The creatinine increased 0.14 mg/dL in the elvitegravir/cobicistat arm and 0.01 mg/dL in the efavirenz arm at 48 weeks. Drug resistance emerged in 8 participants in each arm. In the elvitegravir/cobicistat arm, integrase resistance emerged in 7 participants and nucleoside analogue reverse transcriptase inhibitor (nRTI) resistance in 8 participants. In the efavirenz arm, nonnucleoside analogue reverse transcriptase inhibitor (NNRTI) resistance emerged in 8 participants and nRTI resistance in 2 participants.

DeJesus and colleagues presented data on a clinical trial of similar design and enrollment criteria comparing elvitegravir/cobicistat/tenofovir/emtricitabine with atazanavir/r plus tenofovir/emtricitabine (Abstract 627). This study enrolled 708 participants (90% male; 26% nonwhite). Viral suppression by the FDA snapshot algorithm at week 48 was 90% and 87%, respectively (difference, 3.0%; 95% CI, -1.9-+7.8). Elvitegravir/cobicistat/tenofovir/emtricitabine was found to be noninferior to the atazanavir/r-containing regimen. The mean CD4+ count increases were similar in the 2 arms (207 cells/μL and 211 cells/μL, respectively). Drug discontinuations due to adverse events occurred in 4% and 5% of participants, respectively.

Dolutegravir

Dolutegravir is an investigational InSTI with activity against most isolates resistant to raltegravir or elvitegravir. Investigators presented data on the 96-week outcomes from the SPRING-1 trial: a randomized, partially blinded, dose-finding study of dolutegravir versus efavirenz, each paired with 2 nRTIs (Abstract 102LB). Participants were required to have a plasma HIV-1 RNA level greater than 1000 copies/mL and CD4+ count greater than 200 cells/μL. The 205 participants were randomized to receive either dolutegravir (10 mg, 25 mg, or 50 mg) or efavirenz. The participants were 86% male and 20% nonwhite, with a mean CD4+ count of 343 cells/μL, and mean plasma HIV-1 RNA level of 4.46 $\log_{10}$ copies/mL. At week 96, the plasma HIV-1 RNA level was below 50 copies/mL in 79%, 78%, and 88% of participants for the dolutegravir groups, respectively, compared with 72% for the efavirenz group. No InSTI or NNRTI resistance was detected in any participant. Drug-related grade-2 to -4 adverse events occurred less frequently in the dolutegravir arms than in the efavirenz arm (11% vs 24%), and fewer participants discontinued dolutegravir than discontinued efavirenz for adverse events (3% vs 10%). It is important to note that randomization to dolutegravir versus efavirenz was open-label, and only the dosage of dolutegravir was blinded. The dolutegravir groups overall had an increase in serum creatinine of 0.1 mg/dL to 0.15 mg/dL, which did not progress over time. The investigators noted that this is consistent with a small inhibition of creatinine secretion in the proximal renal tubules. Ongoing clinical trials in ART-naive individuals are using a dosage of dolutegravir 50 mg once daily.

Antiretroviral Therapy Strategies

Lopinavir/r Monotherapy

Du Pasquier and colleagues examined cerebrospinal fluid from participants in the MOST (Monotherapy Switzerland/Thailand) trial, which randomized participants to continued therapy with lopinavir/r and 2 nRTIs or lopinavir/r monotherapy (Abstract 480). The study was stopped early because of viral breakthrough in the monotherapy arm. The researchers examined 5 markers of CNS inflammation from 52 participants who maintained virologic suppression throughout trial participation and

found that 2 of these markers (S100-beta and neopterin) were higher in the monotherapy group (P = .002 and P = .058, respectively). The authors concluded that lopinavir/r leads to CNS inflammation despite virologic control in the plasma.

Adherence Interventions

Two clinical trials evaluated adherence interventions. Lucas and colleagues enrolled 107 participants attending a methadone maintenance program who were either not receiving ART or receiving ART but had ongoing viremia (Abstract 628). Participants were randomized to directly observed ART for one dose per day on weekdays (other doses self-administered) or self-administered ART. The proportion achieving virologic suppression was marginally higher in the direct-observation group: 51% versus 40% at month 12 (difference, 11%; 95% CI, -2%-24%; P = .09).

Investigators from the University of Pennsylvania evaluated a behavioral intervention called managed problem solving (MAPS), a five-step behavioral intervention (Abstract 629). Eligible participants were either ART-naive or restarting ART. The researchers randomized 180 participants, of whom 61% were male, 85% black, and 58% treatment-experienced. The primary endpoint was adherence as measured by a microelectronic monitoring system (MEMS). Adherence was greater in the MAPS group (69% of doses taken compared with 39% for non-MAPS, P = .023). The odds of having an undetectable plasma HIV-1 RNA level were significantly higher for the MAPS group in the as-treated analysis (missing = ignored), and marginally higher in the intent-to-treat analysis (missing = failure).

Clinical Trials to Decrease the Latent Reservoir of HIV-1 Infection

A major obstacle to eradication of HIV-1 is the proviral latency of HIV-1 in resting CD4+ T cells. Histone deacetylase inhibitors have the potential to disrupt this latency to allow clearance of HIV-1 from this reservoir. Margolis and colleagues investigated the effects of vorinostat ex vivo and in vivo (Abstract 157LB). They presented data on 5 men with sustained suppression of plasma HIV-1 RNA on ART. The researchers obtained peripheral blood mononuclear cells (PBMCs) by leukapheresis and confirmed ex vivo that the addition of vorinostat resulted in an increase in HIV-1 RNA expression. Participants received a 400 mg infusion of vorinostat at a subsequent visit. Pharmacokinetic parameters of vorinostat observed in this trial were similar to those obtained in oncology trials. Leukapheresis was performed a second time. HIV-1 RNA levels increased significantly in resting CD4+ T cells with a mean 5-fold change (range, 3-9 fold). This provides proof of concept that HIV proviral latency can be disrupted in vivo and suggests a trial design for evaluating such an approach.

Disulfiram has been shown to induce HIV-1 expression in resting memory T cells in vitro. Spivak and colleagues investigated whether disulfiram would lead to increased plasma HIV-1 RNA levels as measured by the single-copy assay in HIV-1-infected participants with sustained viral suppression (Abstract 369). The researchers enrolled 14 participants who received disulfiram for 2 weeks. The drug was well tolerated with no substantial adverse effects. The researchers observed a nonsignificant increase in plasma HIV-1 RNA levels (difference, 55%; 95% CI, –28%-225%) during disulfiram treatment and afterward (difference, 88%; 95% CI, -23%-355%). This study is ongoing.

Pharmacokinetic Interactions of ART Agents

Drugs and ART Agents

Dooley and colleagues presented data on 11 healthy, HIV-uninfected participants receiving dolutegravir and rifampin (Abstract 148). Dolutegravir is metabolized primarily by UDP-glucuronosyltransferase 1A1 (UGT1A1) and, to a lesser extent, by cytochrome P450 3A4 (CYP3A4). Both of these enzymes are induced by rifampin. The investigators hypothesized that the expected drug-drug interaction could be overcome by increasing the dolutegravir to 50 mg twice daily. Participants received 7 days of dolutegravir dosage 50 mg once daily, followed by 7 days of dolutegravir 50 mg twice daily, followed by 14 days of dolutegravir 50 mg twice daily and rifampin 600 mg once daily. Dolutegravir twice daily with rifampin resulted in dolutegravir concentrations that were 22% to 33% higher than those achieved by once-daily dolutegravir. This strategy effectively overcame the drug-drug interaction, suggesting that dolutegravir-based regimens should be investigated further for HIV-infected patients with active tuberculosis (TB).

ART Pharmacokinetics in Participants with Liver Disease

Dolutegravir pharmacokinetics were investigated in 8 participants with moderate hepatic dysfunction (Child-Pugh score, 7-9) and 8 healthy matched controls (Abstract 608). The study found that the pharmacokinetic parameters were generally similar between groups, with no appreciable difference except for a small increase in the concentrations of non-protein-bound dolutegravir in participants with moderate hepatic impairment.

Other investigators evaluated the pharmacokinetics of raltegravir in 5 hepatitis C-virus (HCV)/HIV-coinfected participants with advanced cirrhosis and 5 with no histologic damage on liver biopsy (Abstract 609). Participants were virologically suppressed for at least 6 months on a regimen containing lopinavir/r, fosamprenavir/r, or darunavir/r. Raltegravir 400 mg was added twice daily for 5 days. Raltegravir concentrations were higher in the group with cirrhosis: the 12-hour area under the curve (AUC) was 1.72 times higher (90% CI, 1.02-2.92), and the trough concentration was 6.58 times higher (90% CI, 2.92-14.85). Despite the higher concentrations, no safety concerns were identified, and raltegravir was well tolerated by all participants.

Inhaled Beclomethasone

Boyd and colleagues investigated the drug interactions with inhaled beclomethasone in 30 HIV-uninfected participants (Abstracts 610 and 611). All participants received inhaled beclomethasone twice daily for 14 days. On day 15, participants were randomized to 1 of 3 groups: inhaled beclomethasone alone twice daily; inhaled beclomethasone and ritonavir 100 mg twice daily; or inhaled beclamethasone, darunavir 600 mg, and ritonavir 100 mg twice daily. Each regimen was administered for 14 additional days. The concentrations of basal morning cortisol and response to adrenocorticotropic hormone stimulation testing did not differ among the groups. The pharmacokinetics of the active beclomethasone metabolite were not altered by darunavir/r. Interestingly, the concentrations of the beclomethasone metabolite were statistically significantly increased by ritonavir alone ($P = .006$), suggesting that ritonavir alone cannot be used to assess the drug-drug interactions for ritonavir-boosted protease inhibitors (PIs).

Antimalarial Drugs

Two abstracts reported on the interaction between malaria treatments and commonly used antiretroviral drugs in HIV-1-infected adults with malaria coinfection. Kredo and colleagues compared lumefantrine and dihydroartemisinin exposure over 72 hours of twice-daily dosing in 18 HIV-infected adults not on ART with 16 HIV-infected adults on lopinavir/r (Abstract 613). The study found that the drug concentrations of dihydroartemisinin were not altered by lopinavir/r. The concentrations of lumefantrine were statistically significantly higher in the lopinavir/r group. However, the increased concentrations were not associated with adverse clinical events or QT prolongation.

Byakika-Kibwika and colleagues assessed the pharmacokinetic interaction of nevirapine and artemether/lumefantrine in HIV-infected adults (Abstract 614). Participants received 6 doses of artemether/lumefantrine and underwent intensive pharmacokinetic sampling. The dosing and sampling process was repeated after dosing nevirapine to steady state. The study found statistically significant reductions in artemether and dihydroartemsinin concentrations with nevirapine coadministration. The levels of lumefantrine were not affected. Concentrations of nevirapine were statistically significantly lower with artemether/lumefantrine coadministration. This study suggested that alternative malaria treatments should be pursued for participants receiving nevirapine.

HCV NS5A Inhibitor

BMS-790052 is an HCV nonstructural protein 5A (NS5A) inhibitor that is entering clinical investigation in HIV/HCV-coinfected individuals. Bifano and colleagues reported on 3 separate clinical trials examining potential interactions of BMS-790052 with tenofovir, efavirenz, and atazanavir/r (Abstract 618). The concentrations of the ART agents did not appear to be changed by coadministration with BMS-790052. Efavirenz decreased the concentrations of BMS-790052, atazanavir/r increased the concentrations of BMS-790052, and tenofovir had no substantial effect. The authors estimated that higher doses of BMS-790052 were necessary when coadministering with efavirenz (90 mg vs 60 mg), and lower doses were necessary when coadministering with atazanavir/r (30 mg vs 60 mg).

Treatment Outcomes and Mortality Data from Secondary Analyses and Observational Cohorts

The conference showcased many interesting presentations using data from large, observational cohorts. See Table 1 for a review of notabel findings. One of the highlights was a presentation by Skarbinski and colleagues (Abstract 138) of data from the US Centers for Disease Control and Prevention (CDC) Medical Monitoring Project (MMP). The MMP collects data from individual HIV-infected adults receiving care at a sample of outpatient HIV-care facilities within 17 states or territories that contain 76% of all persons diagnosed with HIV in the United States. Interview and medical-record data are collected and linked to the National HIV Surveillance system, and population-size estimates are calculated using weighting for design and nonresponse adjustments.

The researchers collected data from 4217 patients receiving care at 461 facilities between January and April of 2009. Patient and facility response rates were 53% and 76%, respectively. Using a weighted analysis, the researchers estimated that 421,186 HIV-seropositive adults received at least 1 visit in an HIV clinic during the observation period. This represented 44% of the estimated 941,950 adults living with HIV. Of the patients in care, 89% (95% CI, 87%-91%) were prescribed ART, and 71% (95% CI, 68%-75%) had an HIV-1 plasma RNA level of 200 copies/mL or below. Adjusted, multivariate logistic regression models were developed to predict ART prescription and virologic suppression. Non-Hispanic blacks were less likely to achieve both than were non-Hispanic whites. The authors concluded that, because fewer than half of HIV-seropositive individuals were estimated as being in care, expanding ART coverage for those with CD4+ counts greater than 500 cells/μL would have a minor impact. Increasing the number of HIV-infected persons in care is essential to reduce mortality and impact transmission.

Four presentations addressed issues of mortality in HIV-infected cohorts in western and northern settings (see Table 1). Hogg and colleagues (Abstract 137) used data from 18 clinical and interval cohort studies in the United States and Canada that participate in NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design). The analysis estimated changes in life expectancy between 1996 and 2007 for HIV-infected individuals 20 years of age or older who were ART naive and initiating ART. Death ascertainment occurred through direct linkage to vital-statistic registries and clinic reports, and death and person-time were partitioned into 5-year groups. Life expectancy from age 20 years

increased steadily, from 34.4 years in the 1996-to-1999 period to 47.1 years in the 2006-to-2007 period. The life expectancy of injection drug users (IDUs) was lower (28.1 years) than it was for either men who have sex with men (MSM) (51.6 years) or those who acquired HIV through heterosexual sex (47.7 years). The gap between average national life expectancy at age 20 years in the general US population and that in the cohort was largest for African Americans at 54.7 years versus 41.0 years, respectively, compared with 59 years versus 50 years for whites. Finally, life expectancy by pre-ART CD4+ count was lower for those with a count below 100 cells/μL (18.7 years; standard error [SE], 0.7 years) than for those with a count of more than 350 cells/μL (42.2 years; SE, 0.5 years). The authors concluded that life expectancy of an HIV-infected person on ART would be only slightly lower than that of one in the general population, with notable differences by race and HIV risk factor.

Rodger and colleagues undertook a similar analysis (Abstract 638) to compare mortality rates in adults with well-controlled HIV with those of adults in the general population. The researchers used data from the SMART (Strategies for Management of Antiretroviral Therapy) and ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) studies to examine mortality in 3280 participants who met inclusion criteria of being from 20 years to 70 years old, receiving continuous ART, not being IDUs, and having HIV-1 plasma RNA levels below 500 copies/mL and CD4+ counts of 350 cells/μL or higher at any time in the previous 6 months. Standardized mortality ratios (SMRs) were calculated by comparing death rates with those in the Human Mortality Database (HMD), after stratification by country, sex, and age. For participants with a CD4+ count between 350 cells/μL and 499 cells/μL, the SMR was 1.79 (95% CI, 1.19-2.59), reflecting an increased mortality rate compared with the general population. However, for participants with CD4+ counts of 500 cells/μL or higher, there was not a statistically significant increase in mortality compared with the general population. The most common cause of death overall was cardiovascular disease (CVD) or sudden death (19 deaths, 31%), followed by non–AIDS-related malignancy (12 deaths, 19%). These results were robust to 2 separate sensitivity analyses, but the authors cautioned that a selection bias of healthier individuals may have been present in these 2 clinical trials.

Morlat and colleagues compared causes of death in a cross-sectional, nationally representative 2010 survey of 90 HIV treatment sites in France (representing approximately 82,000 patients) with causes of death found in similar surveys in 2000 and 2005 (Abstract 1130). The proportion of deaths due to AIDS-defining events decreased from 47% in 2000 to 36% in 2005 and to 25% in 2010, but AIDS-defining events remained the most frequent cause of death overall. Conversely, the proportion of non–AIDS-defining and non–hepatitis-related malignancies increased from 11% in 2000 to 22% in 2010. Cardiovascular deaths were similar in proportion in the 3 time periods at 7% in 2000, 8% in 2005, and 10% in 2010. The authors emphasized the need for integrated care, with prevention, screening, and treatment for malignancies and viral hepatitis, as well as continued improvements in HIV control.

In the last presentation addressing mortality in ART cohorts, Ingle and colleagues from ART-CC (Antiretroviral Therapy Cohort Collaboration) examined cause-specific mortality in 16 cohorts containing 65,121 HIV-infected people from Europe and North America (Abstract 640). The overall mortality rate was 12.9 per 1000 person-years, and a specific cause could be determined for 84% of the deaths. Of the overall mortality, AIDS-related deaths accounted for 42%, and non-AIDS-related deaths accounted for 58%. By using Poisson regression to estimate crude and adjusted mortality rates by ART duration, AIDS-related deaths were found to decrease significantly with each year on ART (adjusted rate ratio [aRR], 0.81/year; 95% CI, 0.79-0.83). Rates of death from both non–AIDS-related malignancy and stroke increased significantly (aRRs, 1.05; 95% CI, 1.01-1.09, and 1.13; 95% CI, 1.02-1.26, respectively). Similar to what was seen in prior presentations, non–AIDS-related mortality surpassed that from AIDS after 2 years on ART, led by non-AIDS-related malignancies and CVD risks. This again highlighted the need for HIV-specific care to integrate longitudinal prevention and treatment for malignancies of all types and CVD.

Two other cohort studies found health disparities by race, one examining CD4+ cell count at ART start (Abstract 139) and one determining AIDS as a cause of death (Abstract 1045). The first presentation, by Troung and colleagues, assessed CD4+ cell count at ART initiation in the San Francisco HIV/AIDS case registry for individuals older than 12 years diagnosed with HIV infection between 2004 and 2010 (Abstract 139). In 2010, public health clinics in San Francisco began recommending ART at any CD4+ cell count. The investigators found consistent increases in CD4+ cell count at ART initiation from 2004 to 2010. Early initiation of ART, at CD4+ counts greater than 500 cells/μL, was seen starting in 2007. However, after adjustment for the number of people presenting in each substratum, people initiating ART at CD4+ counts greater than 500 cells/μL were more likely to be white, MSM, non-poor, and diagnosed by a private practitioner ($P < .01$ for each association, compared with all others in group). The authors speculated that these disparities, if carried forward, could partition the benefits of initiating ART at CD4+ counts greater than 350 cells/μL or 500 cells/μL into less marginalized populations.

A second presentation, by Murphy and colleagues, examined the association between black race and death from AIDS in the WIHS (Women's Interagency HIV Study) cohort (Abstract 1045). Inclusion criteria were enrollment in the WIHS cohort and continuous ART, primary exposure was self-reported race, and the primary outcome was AIDS-related death. In the univariate

Table **1**. Selected Studies on Treatment Outcomes and Mortality in People Receiving Antiretroviral Therapy in Resource-Rich Settings

Study Description	Cohort Description	Key Findings
Abstract 138 US-based, nationally-representative estimate of HIV+ adults in care, on ART, and virologically suppressed	Structured sample of 4217 patients at 461 facilities in 17 states or territories Observation period: Jan–April 2009 Using weighted analysis, this was representative of 421,186 HIV+ adults in care	• 44% of an estimated 941,940 adults living with HIV were in care (had 1 clinic visit during observation period). Of those in care: - 89% were prescribed ART - 71% had HIV-1 plasma RNA level ≤200 copies/mL • Non-Hispanic blacks were less likely to be prescribed ART and be virologically suppressed in adjusted MVA *Conclusion:* Because < half of HIV+ individuals were in care, expanding ART coverage to those with CD4+ cell counts >500 cells/μL will have minor impact. Increasing numbers in care is important.
Abstract 137 NA-ACCORD analysis to estimate temporal changes in life expectancy between 1996 and 2007	18 clinical and interval cohorts in U.S. and Canada Observation period: 1996–2007	• Life expectancy at age 20 increased: - 1996-1999: 34.4 years - 2006-2007: 47.1 years • Life expectancy at age 20 was lower for IDU (28.1 years) than for MSM (51.6 years) or heterosexuals (47.7 years) • Gap between life expectancy at age 20 in HIV+ vs general population: - Blacks: 41 years (HIV+) vs. 54.7 years (general population) - Whites: 50 years (HIV+) vs. 59 years (general population) *Conclusion*: Life expectancy at 20 years of an HIV+ person on ART is only slightly lower than that of the general population, but notable differences exist by race and HIV risk factor.
Abstract 638 Mortality in patients with well-controlled HIV and high CD4+ cell counts in 2 randomized controlled trials, compared with the general population	3280 non-IDU participants in the ESPRIT or SMART studies with CD4+ ≥350 cells/μL and HIV-1 plasma RNA <500 copies/mL were randomized to the continuous ART arms Observation period: 2000–2008 [ESPRIT], 2002–2006 [SMART] SMRs calculated comparing death rates in the combined cohort with those in the Human Mortality Database, stratifying by country, sex, and age	• SMR for participants with CD4+ count between 350 and 499 cells/μL: - 1.77; 95% CI, 1.17-2.55 • Standardized mortality ratio for participants with CD4+ count >500 cells/μL: - 1.00; 95% CI, 0.69-1.40 • Most common causes of death: - Cardiovascular disease or sudden death (n=19 or 31%) - Non–AIDS-defining malignancy (n=12, 19%) *Conclusion:* Risk of mortality in healthy HIV+ patients on ART enrolled in 2 clinical trials was not statistically significantly higher than that for the general population, though a selection bias toward healthier trial participants may be present.
Abstract 1130 Causes of death among HIV+ patients in France from 2000 to 2010	Cross-sectional, nationally representative survey of 90 HIV treatment sites in France, ~82,000 patients [ANRS Cohort] Observation period: cross-sectional surveys conducted in 2000, 2005, 2010	• Proportion of deaths from AIDS-defining events decreased from 47% in 2000 to 25% in 2010 • Proportion of non–AIDS-defining and non–hepatitis-related malignancies rose from 11% in 2000 to 22% in 2010 • Proportion of cardiovascular deaths not statistically significantly different: 7% in 2000, 10% in 2010 *Conclusion:* AIDS-defining events have decreased, but still account for 1/4 of all mortality. Integrated care with prevention, screening, and treatment for malignancies, hepatitis, and cardiovascular disease will be increasingly important.
Abstract 640 Cause-specific mortality in European and North American cohorts	Longitudinal data from the ART Cohort Collaboration assessed mortality for 65,121 HIV+ people in 16 cohorts Observation period: 1996–2009	• Overall mortality rate was 12.9 per 1000 person-years • Specific cause of death determined for 84% of individuals: - 42% were AIDS-related - 58% were non–AIDS-related • AIDS-related deaths decreased each year on ART: - Adjusted rate ratio 0.81/year; 95% CI, 0.79-0.83 • Non–AIDS-related mortality surpassed that from AIDS after 2 years on ART: - Non–AIDS-related malignancy increased aRR/year on ART: 1.05; 95% CI, 1.01-1.09 - Stroke increased aRR/year on ART: 1.13; 95% CI, 1.02-1.26 *Conclusion:* Integrated HIV care should address HIV-specific factors, eg, malignancies and cardiovascular risk.

HIV+ indicates HIV seropositive; ART, antiretroviral therapy; MVA, multivariable analysis; IDU, injection drug users; CI, confidence interval; MSM, men who have sex with men; NA-ACCORD, North American AIDS Cohort on Research and Design; ESPRIT, Evaluation of Subcutaneous Proleukin in a Randomized International Trial; SMART, Strategies for Management of Antiretroviral Therapy Study Group; SMR, standard mortality ratio; ANRS, French National Agency for Research on AIDS and Viral Hepatitis; aRR, adjusted rate ratio

model, the hazard ratio (HR) for death from AIDS was 2.11 for black women compared with white women (P = .002); the difference remained statistically significant after adjustment for HCV, IDU, depression, and virologic control. Black women were also less likely than white women to adhere to ART (adjusted HR [aHR], 0.65; P = .0001), but race remained a predictor of death from AIDS after adjustment for adherence. The authors suggested that further studies are needed to determine the role of genetic polymorphisms that vary by race in lower adherence and response to treatment among black women.

Acute HIV Infection

Several studies were dedicated to understanding the effect of ART on acute HIV infection. To further elucidate the possible benefits of ART during acute or early HIV infection, Margolick and colleagues reported on the effect of ART on rates of viral suppression at 24 months off therapy for patients with acute or early HIV infection enrolled from 2005 to 2009 (Abstract 356). Patients with acute or early HIV infection and CD4+ count of 350 cells/µL or higher were randomized to 12 months of immediate ART (n = 57) or deferred treatment (n = 56), and both groups were followed for 24 months off therapy. The plasma HIV RNA set point at 24 months in those who had not (re)initiated ART was below 10,000 copies/mL in 17.5% of patients in the immediate-treatment arm and 5.4% of the patients in the deferred-treatment arm (P = .07). Similarly, Lafeuillade and colleagues reported on the long-term control of HIV after a 2-year ART course for acute HIV infection in 45 patients (Abstract 358). Of patients who had received a 2-year ART course initiated at the time of acute infection, 17% were still off therapy 12 years after completing the course. The majority of those patients demonstrated stable plasma viral levels of less than 3500 copies/mL.

Perelson explored the impact of ART during acute infection on the level of resting CD4+ cell infection and decay of latent infection (Abstract 152). Investigators developed a mathematical model of the initial frequency of resting-cell infection and decay of latent infection and compared the model with T-cell and HIV RNA-level data from 27 study participants who elected to start ART during acute HIV infection. The model predicted a strong relationship between containment of viremia and the level of resting-cell infection (r = 0.65, P = .0003). The authors suggested that there may be 2 pools of infected resting CD4+ T cells: a less stable pool of cells observed in patients with high levels of resting-cell infection and an extremely stable pool of cells that are established despite ART. The less stable pool may be easily activated and eliminated, but the stable pool may have an extremely long half-life and be highly resistant to elimination, even by long-term ART.

Buzon and colleagues presented findings on patients who initiated ART during early HIV infection and continued on ART for more than 10 years, compared the effect on the viral reservoir with elite controllers (Abstract 151). Investigators compared CD4+ cell counts and levels of total, integrated, and 2-long terminal repeat (LTR) HIV-1 DNA by quantitative polymerase chain reaction (PCR) in 9 individuals treated during acute HIV infection, 26 individuals with established HIV on treatment, and 37 elite controllers. Levels of integrated and total HIV-1 DNA were statistically significantly lower in elite controllers and in patients who began treatment during acute infection (P = .06 and P = .001, respectively). The ratio between total and integrated HIV-DNA was statistically significantly lower in patients treated early than in chronically treated patients (P = .04) and elite controllers (P = .04). The authors suggested that this difference in ratio is evidence that prolonged ART initiated during acute infection may allow for a clinically significant depletion of HIV-1 reservoirs.

Hamlyn and colleagues explored factors associated with progression after primary HIV in participants of the SPARTAC (Short Pulse Anti Retroviral Therapy at HIV Seroconversion) Trial (Abstract 553). SPARTAC participants were randomized to 1 of 3 arms: no treatment, 12 weeks of ART, or 48 weeks of ART. The primary study endpoint was either CD4+ count less than 350 cells/µL or initiation of ART. The authors explored associations between interleukin-6 (IL-6) and D-dimer levels at HIV seroconversion as predictors of HIV disease progression. Data on IL-6 and D-dimer were available for 200 patients. There was no association between D-dimer level and progression; however, IL-6 levels at HIV seroconversion were independently associated with HIV disease progression even after controlling for HIV RNA levels and CD4+ cell counts (HR, 1.38; 95% CI, 1.09-1.75; P = .007). The authors urged further exploration of the IL-6 marker.

Vinikoor and colleagues analyzed immunologic outcomes of acutely infected HIV patients who started ART within 30 days of diagnosis in a prospective clinical trial with a primary outcome of T-cell activation as measured by percent of circulating CD8 + HLA-DR + CD38 + T cells at 96 weeks (Abstract 554). Thirty-one patients were included in the 96-week analysis. In multivariable analysis, adjusted for age, nadir CD4+ cell count, and peak HIV RNA level, there was no association between the length of time from infection to ART initiation and immune activation at 96 weeks (P = .8).

To understand the impact of ART regimens on the viral load in the anogenital compartment, Phanuphak and colleagues compared 3-drug (tenofovir/emtricitabine/efavirenz) with 5-drug (tenofovir/emtricitabine/efavirenz/raltegravir/maraviroc) ART regimens in 24 men with acute HIV infection (Abstract 555). Investigators collected blood, anal lavage, and seminal plasma to measure HIV RNA levels from baseline to 24 weeks on treatment. For anal lavage, median time to HIV RNA levels less than 3 $\log_{10}$ copies/mL and less than 1.7 $\log_{10}$ copies/mL was 4 days (3 days-13 days) and 3 days (3 days-7 days), respectively. For seminal plasma, the median times to these levels were 7 days (3 days-26 days) and 12 days (6 days-26 days),

respectively. There was no difference between the ART regimens in time to achieve HIV RNA level less than 3 $\log_{10}$ copies/mL and less than 1.7 $\log_{10}$ copies/mL in anal lavage (P = .33 and P = .92, respectively) or in seminal plasma (P = .90 and P = .08, respectively). The 5-drug regimen and a high baseline HIV RNA level on anal lavage were both statistically significantly associated with a greater likelihood of a serum HIV RNA level lower than 1.7 $\log_{10}$ copies/mL at 24 weeks. The authors encouraged uptake of early treatment to decrease the risk of transmission.

Ananworanich and colleagues described the effect of 5-drug and 3-drug highly active antiretroviral therapy (HAART) regimens during acute HIV infection on restoration of immunity and HIV reservoir size in 35 patients (Abstract 363). Twenty-two patients were treated with a 5-drug combination (tenofovir/emtricitabine/efavirenz/raltegravir/maraviroc) and 13 were treated with a 3-drug combination (tenofovir/emtricitabine/efavirenz). HIV RNA and DNA levels were measured in the blood and sigmoid colon at baseline and at 24 weeks, and PBMCs were analyzed by flow cytometry. After 24 weeks of treatment, HIV RNA and total and integrated DNA in the PBMCs all declined significantly, whereas in the sigmoid colon, a statistically significant decline was seen only for HIV DNA levels after 5-drug therapy. Similar results were seen when HIV DNA levels were adjusted for CD4+ cell count. At week 24, overall total DNA was undetectable in blood in 8 of 27 patients and integrated DNA was undetectable in blood in 18 of 24 patients. In the sigmoid colon, total DNA was undetectable in 5 of 14 patients and integrated DNA in 7 of 10 patients. Predictors for higher total DNA level in blood at week 24 were higher baseline total DNA and use of 5-drug regimen. Authors concluded that both 5-drug and 3-drug antiretroviral regimens during acute HIV reconstitute CD4+CCR5+ T cells in the gut and reduced HIV reservoir in the blood and gut. Additionally, the 5-drug regimen may have a greater impact on the HIV reservoir in the gut than the 3-drug therapy.

Pérez-Santiago and colleagues reported a study aimed at understanding perturbations and recovery of gut flora during acute HIV infection and the effect of ART on these processes (Abstract 546). Investigators found statistically significant associations between CD4+ count and specific gut bacterial flora during HIV acute infection. Seven subjects with acute infection underwent anal swab, lymphocyte subset profiles, and viral HIV RNA testing at baseline and at weeks 24 to 36. Six patients were treated with ART, and 1 was not. Researchers employed pyrosequencing of the 16s ribosomal DNA (rDNA) to evaluate the gut flora and to characterize associations of bacterial populations with immune status and treatment. Proportions of bacterial populations did not differ significantly between time points among treated subjects. Five orders of bacteria were positively correlated with CD4+ cell count, and 2 orders were negatively correlated. Bacterial population changes were greatest in the single ART-naive individual. The researchers concluded that proportions of gastrointestinal populations shift during acute HIV infection, but ART appears to stabilize gut flora, and untreated acute infection results in the greatest perturbation.

Detection of acute HIV infection is crucial but often overlooked in clinical settings. O'Connell and colleagues presented data from a prospective screening study that showed particularly subtle clinical features of acute HIV. The ECHO (Early Capture HIV Cohort) study enrolled individuals in East Africa and Thailand at high risk for HIV infection for prospective observation (Abstract 60LB). Study participants underwent a baseline history and physical followed by routine clinical follow-up and twice-weekly nucleic-acid testing (NAT). Individuals who became infected with HIV were evaluated over 9 subsequent visits for symptoms, physical-exam findings, and immunologic or virologic parameters. This analysis included 32 volunteers with evidence of acute HIV infection. Investigators reported that clinical symptoms were mild and occurred just before and during the peak of viremia. The most common symptoms were subjective fever (in 50% of participants) and headache (in 38%). Physical examination detected abnormalities in 84% of participants, and lymphadenopathy was the most common finding. Interestingly, fever was documented in only 1 participant at 1 visit. Hospitalization was also notably rare, having occurred in only 2 pregnant women. The authors emphasized the limitations of using clinical manifestations to identify acute HIV infection.

Lodi and colleagues categorized patients in the CASCADE (Concerted Action on Seroconversion to AIDS and Death in Europe) cohort using 3 different definitions of severe primary HIV infection (PHI) and compared rates of survival based on each definition (Abstract 550). Acutely infected patients were considered to have severe or non-severe PHI based on the following 3 definitions: (1) clinical: symptomatic seroconversion illnesses with brain and neurological involvement; (2) immunologic: at least 1 CD4+ count less than 350 cells/µL in the first 6 months; or (3) virologic: at least 1 HIV-1 RNA level over 1,000,000 copies/mL in the first 6 months. For each definition, Kaplan-Meier curves and log-rank tests were used to compare survival (all-cause mortality) for severe and non-severe PHI during follow-up without ART ending at the earliest of 3 events: death, last clinic visit, or January 1, 1997. Of 3878 individuals with acute HIV infection and a recorded CD4+ count, 919 (24%) had severe PHI based on the immunologic definition. There was strong evidence of difference in survival by severity of PHI (P < .001) with median interquartile range (IQR) survival times of 7 years (5 years to 10 years) and 13 years (7 years to -) for severe and non-severe PHI, respectively. There was no statistically significant difference in survival between patients with and without reported severe clinical illness (P = .667) or based on the virologic definition (P = .338). The authors concluded that a low initial CD4+ count may be a useful indicator for identifying patients who are likely to experience rapid disease progression.

Yue and colleagues explored the cumulative impact of host and viral factors on HIV-1 RNA control after HIV-1 transmission (Abstract 551). A heterosexual Zambian transmission cohort provided 195 phylogenetically linked HIV-1 transmission pairs. HLA class I (HLA-I) and HIV-1 viral set point analysis was performed for both partners and associations were determined based on univariate and multivariate analyses. Viral load set point in the source partner was significantly correlated with the viral load set point in the seroconverter (Pearson Correlation R2 = 0.020; P = .046). However, this effect was significantly modulated by sex, HLA-I marker, and HLA-B allele. Multivariable general linear regression analysis accounting for these factors continued to reveal a strong association between transmitting partner viral load and seroconverter viral load (beta = 0.28 $\log_{10}$ viral load; P < .001).

Session 102 was dedicated to diagnostic tools for detecting acute HIV. Peel and colleagues shared an evaluation of the APTIMA® HIV-1 Qualitative RNA Assay (Gen-Probe) for identification of acute HIV infection as a screening assay in high-risk populations in East Africa and Southeast Asia (Abstract 556). A total of 48,229 results were analyzed from 1045 participants. Of these, 127 patients tested reactive by the APTIMA®, with 18 incident cases detected. Prevalence among the high-risk populations examined was 15.6% (Thailand), 12.4% (Tanzania), and 10.1% (Kenya). Infection status over time was used to calculate the sensitivity, which was 99.2%, and specificity, which was 99.3%. Karris and colleagues assessed the cost of missed acute HIV diagnoses in a comparative analysis of an HIV NAT and a fourth-generation antigen/antibody combination assay (ARCHITECT® HIV Combo) for detection of acute HIV performed retrospectively in blinded, banked samples (Abstract 557). A negative rapid test and a positive NAT detected 14 cases of acute infection. Of these, the fourth-generation combo assay detected 9 cases, suggesting that the fourth-generation combo assay had 5 false-negative results. The investigators predicted these 5 missed diagnoses would result in 4.55 new transmissions over 12 months, compared with fewer transmissions when HIV serostatus is known. Cost-savings analysis was performed, and the authors concluded that the NAT should be used in populations with a high incidence of acute HIV infection.

High viral loads associated with acute HIV infection raise the risk of transmission. Kuruc and colleagues found that acute HIV infections detected through the North Carolina Screening and Tracing Active Transmission (STAT) program showed an increasing proportion of infections in African Americans, MSM, and individuals less than 30 years (Abstract 566). The researchers encouraged rapid assessment of acute HIV infection cases so that patients could enter care and begin treatment immediately to avoid transmitting the virus. Frange and colleagues reported on the increasing role of primary HIV-1 infection in the spread of HIV in the ANRS (French National Agency for Research on AIDS and Viral Hepatitis) PRIMO (Primary Infection) cohort in France (Abstract 1107). Investigators analyzed the HIV *pol* sequence using a phylogenetic approach to characterize HIV transmission dynamics in 987 patients. An increasing frequency of primary HIV cosegregated transmission chains was identified, with 10.2% before 2006 and 15.2% from 2006 to 2010. Compared with unique primary HIV, clusters of primary HIV were more often MSM, of younger age, and having reported more casual partnerships. With this in mind, the authors urged reconsideration of current HIV prevention methods and testing programs.

Advances in ART in RLS

This year's CROI featured many outstanding presentations describing advances in ART in resource-limited settings (RLS). The conference began its coverage of issues in RLS with the 6th Annual N'galy-Mann Lectureship, awarded to Quarraisha Abdool Karim and Salim Abdool Karim, who gave a combined lecture on their experience conducting HIV research in South Africa over the past 3 decades (Abstract 17). The awardees attributed their continued success to a dedication to collaborating with and training researchers and medical students, a willingness to conduct research within the community, and outstanding mentorship.

Scale-up of ART and Response to Treatment in RLS

Bendavid described the impact of HIV development assistance on adult mortality in sub-Saharan Africa as "one of the greatest global health achievements in recent memory" (Abstract 85). He noted the correlation between the rise in HIV assistance to sub-Saharan Africa, from US $75 million annually before 2001 to more than US $5 billion annually by 2004, and the stabilization of and decrease in numbers of deaths from HIV in South Africa. To examine the possible linkage between these 2 trends, Bendavid and colleagues conducted 2 analyses: one to determine if assistance from the President's Emergency Plan for AIDS Relief (PEPFAR) was associated with reductions in all-cause adult mortality, and one to compare changes in all-cause adult mortality associated with PEPFAR with changes in HIV-related deaths associated with PEPFAR. This strategy allowed for the assessment of unintended harms that could be caused by PEPFAR's focus on the HIV epidemic crowding out other health priorities, which could result in a less dramatic reduction in all-cause mortality than what would be expected as HIV-related mortality declines.

Data from the Demographic and Health Surveys (DHSs), cross-sectional, nationally representative surveys conducted annually in many African countries from 1998 to 2008, were pooled from 27 countries, 9 of which were PEPFAR focus countries. Focus countries were statistically significantly more populous than non-PEPFAR focus countries but did not have statistically significant differences in gross domestic product (GDP) per capita or HIV prevalence. In the first analysis, the authors found an unadjusted odds

ratio (OR) for adult death of 0.80 (95% CI, 0.68-0.95) in PEPFAR focus countries compared with nonfocus countries. This association remained statistically significant after adjustment for country-specific factors of HIV prevalence, non-PEPFAR health assistance, GDP per capita, government effectiveness, and individual-level covariates of age, residence in an urban area, and level of education (adjusted OR [aOR], 0.84; 95% CI, 0.72-0.99).

For the second analysis, the authors compared the number of all-cause deaths averted in the 9 PEPFAR focus countries from 2004 to 2008, an estimated 740,800 deaths (95% CI, 443,300-1,808,500), with the number of HIV-specific deaths averted over the same time frame in the same countries, an estimated 631,338 deaths (95% CI, 249,026-1,060,253). The wide range in CIs prevents conclusions about the likelihood of unintended harms. Bendavid and colleagues argued that, despite the uncertainty, the overall effect of PEPFAR intervention on mortality outcomes was beneficial.

Two presentations offered different views of the change in CD4+ cell count at the initiation of ART. Egger and colleagues examined data from patients participating in IeDEA (International Epidemiologic Databases to Evaluate AIDS) and ART-CC who had a CD4+ cell count measured 6 months prior to or 1 month after ART initiation (Abstract 100). The researchers used 2 random-effects linear regression model analyses to examine trends in CD4+ cell count at ART initiation in low-, middle-, and high-income countries. A univariate analysis determined median CD4+ cell count at ART start in 2009 for 36 countries with a collective 244,953 ART patients, and change in CD4+ cell count from 2002 to 2009 or 2010. A multivariable analysis used data from 240,515 patients in 22 countries with more than 500 ART patients each and more than 1 treatment site, and to explore the influences of age, sex, country income, country-level ART coverage, and calendar year on CD4+ cell count at ART start.

The analyses found large discrepancies in median CD4+ cell count at ART initiation in 2009, varying from 89 cells/μL (95% CI, 69-110) in Indonesia to 307 cells/μL (95% CI, 301-314) in the United States. However, trends in median CD4+ cell counts at ART start from 2002 to 2010 were similar in low-income and upper-middle-income countries, with annual changes being largest (20-25 cells/μL/year) in low-income countries. Men and subjects over 40 years of age had lower CD4+ cell counts at ART initiation than other groups. For low-income countries only, national ART coverage for 80% or more of people qualifying for ART by the WHO 2010 guidelines was statistically significantly associated with an annual increase in CD4+ cell count at start of ART, but countries with ART coverage for less than 80% of people diagnosed with HIV did not demonstrate statistically significant changes in CD4+ cell count at ART start. The authors noted limitations in the analyses, including the exclusion of 25% of patients in the cohort because of missing CD4+ cell count data, limited or unrepresentative data from some countries, and the observational nature of the study. The researchers observed that, although median CD4+ count at ART start increased in most countries over time, it remained below 200 cells/μL and 350 cells/μL in low- and high-income countries, respectively. The authors also noted that higher national ART coverage led to statistically significant increases in CD4+ cell counts over time.

Lahuerta and colleagues used data from 151 sites supported by ICAP (International Center for AIDS Care and Treatment Programs) in sub-Saharan Africa with 262,638 eligible patients over 14 years of age to study late enrollment into care and late ART initiation, defined as CD4+ count less than 100 cells/μL or World Health Organization (WHO) stage IV disease (Abstract 650). The researchers found that the proportion of patients enrolling in care late decreased statistically significantly from 20% in 2005 to 16% in 2010 (*P* value for trend < .001). Late ART initiation also decreased from 43% to 31% over the same period (*P* value for trend < .001), and men had higher risk than women of both enrolling late (relative risk [RR], 1.7; 95% CI, 1.6-1.8) and initiating ART late (RR, 1.4; 95% CI, 1.3-1.5) in 2010; there were similar discrepancies in 2005. Median CD4+ count within the cohort rose from 125 cells/μL in 2005 to 178 cells/μL in 2010 (P value for trend < .001), approaching prior WHO thresholds for ART initiation of 200 cells/μL. The authors suggested that targeted efforts are needed to reach HIV-infected men.

To address the lack of information on ART outcomes in women in RLS, Firnhaber and colleagues used data from the AIDS Clinical Trial Group (ACTG) PEARLS (Prospective Evaluation of Antiretrovirals in Resource Limited Settings) trial to examine sex differences in ART efficacy outcomes (Abstract 89). The study randomly assigned 1571 ART-naive participants (47% of whom were women) with CD4+ counts less than 300 cells/μL to receive (A) lamivudine/zidovudine plus efavirenz, (B) didanosine, emtricitabine, and atazanavir, or (C) emtricitabine/tenofovir plus efavirenz. The study ran from May 2005 to 2010, and the primary endpoint was time to treatment failure, defined as confirmed HIV-1 plasma RNA greater than or equal to 1000 copies/mL at week 16 or later, a new AIDS-defining condition (ADC) at week 12 or later, or death due to any cause. A Data Safety and Monitoring Board (DSMB) review in 2008 led to the discontinuation of arm B because of inferior efficacy. The authors used a Kaplan-Meier analysis to examine time to treatment failure by sex and treatment arm, and they used a Cox proportional hazard model to examine the association of sex with outcome in both univariate and adjusted analyses. At baseline, women had a statistically significantly higher body mass index (BMI) and CD4+ cell count than men had, and lower plasma HIV-1 RNA levels (*P* < .001 for all data). In the primary analysis, there was no difference in time to treatment failure between men and women. Interestingly, there was no difference between efficacy of treatment arms in the women in the Kaplan-Meier analysis, but men reached treatment failure more quickly

in the atazanavir-based arm (arm B) than women, and this difference was due to virologic failure. Men were found to report any nonadherence more frequently than women did (42% vs 38%, respectively; $P < .0011$). The data are currently being analyzed to explore these adherence differences as well as possible sex-based pharmacokinetic differences that could explain the poor treatment outcome for men in arm B.

Treatment outcomes in women were also examined by Sawe and colleagues (Abstract 642) using posttrial follow-up data from the ACTG A5208 OCTANE (Optimal Combination Therapy After Nevirapine) study in Africa. In OCTANE, 214 women who had received single-dose nevirapine (sdNVP) for prevention of mother-to-child transmission (PMTCT) and 500 women who had not been exposed to sdNVP initiated ART with tenofovir/emtricitabine plus either lopinavir/r or nevirapine. Of 513 women in follow-up at completion of the study, 77% consented to extended follow-up and transitioned to standard care at local clinics. Of those, 489 (95%) completed 72 weeks of follow-up. No statistically significant increase in grade 3 or 4 adverse events, new HIV-related diagnoses, or WHO clinical stage was seen after transfer to local ART care, compared with such developments while the women were under the care of the study team. These are encouraging results for patients transitioning from care under clinical trials to standard care in RLS, although data are limited because 33% of the patients did not enroll in long-term follow-up.

Clumeck and colleagues presented data from a prospective, randomized clinical trial taking place in the Democratic Republic of Congo (Abstract 88LB). The trial examined differences in treatment outcomes in 425 participants randomized to either lopinavir/r-based or nevirapine-based regimens, with a second tier of randomization to determine nRTI allocation of either tenofovir/emtricitabine or zidovudine/lamivudine. Inclusion criteria included being at least 18 years of age and ART naive (except for sdNVP if given more than 1 year prior to enrollment) and meeting national guidelines for ART initiation (WHO clinical stage IV, WHO clinical stage III with CD4+ count < 350 cells/μL or CD4+ count < 200 cells/μL). Patients with active TB, pregnancy, or substantial laboratory abnormalities were excluded, and there were no statistically significant differences in baseline characteristics among the groups. The primary outcome was therapeutic failure, defined as new categorization of WHO stage III or IV, plasma HIV-1 RNA level greater than 1000 copies/mL, or treatment change because of toxicity. Therapeutic failure was seen in 36 (17%) of the nevirapine-based regimen arm and 23 (11%) of the lopinavir/r-based arm at 96 weeks ($P = .014$). The difference was driven by increased virologic failure in the nevirapine arm. Of those in whom nevirapine failed, 86% had drug resistance mutations, compared with 20% in the lopinavir/r arm, but the statistical significance of this difference was not reported. There was no statistically significant difference in glomerular filtration rate or anemia based on nRTI regimen component. The authors concluded that the increased incidence of virologic failure and resistance seen in the nevirapine-based regimen was of concern and should be taken into account in rollout program planning.

Two other groups presented concordant results regarding the use of regimens containing nevirapine and tenofovir. Wandeler and colleagues (Abstract 635) examined data from the IeDEA Southern Africa Collaboration and found that patients on first-line regimens containing nevirapine and tenofovir were more likely to die (aHR 1.24; 95% CI, 1.15-1.34) but less likely to be lost to follow-up (aHR, 0.86; 95% CI, 0.83-0.90) than those receiving ART containing efavirenz and tenofovir. Scarsi and colleagues (Abstract 636) found a higher initial and persistent rate of virologic failure in Nigerians initiating ART with tenofovir, emtricitabine, and nevirapine (10.6%) than in those initiating with zidovudine, lamivudine, and nevirapine (7.1%, $P < .001$). These results add to the growing call for an evaluation of initial regimens containing nevirapine and tenofovir.

Linkage to Care and Loss to Follow-Up in Adults in RLS

A themed discussion included several presentations on novel approaches to linkage to care. See Table 2 for some of the major findings. McNairy moderated the session, which began with a brief overview, highlighting data from RLS showing that 59% of HIV-infected people link to care, but the range in studies is from 33% to 88%.[1] As linkage to care is not a PEPFAR indicator or routinely reported, data are often limited. McNairy discussed several novel approaches to linkage to care, including point-of-care CD4+ cell count measurements, case management, and financial incentives for care entry. Chamie and colleagues explored the first of these strategies by offering community-based voluntary counseling and testing along with point-of-care CD4+ cell count testing and rapid TB testing during a 5-day multi-disease community health campaign in Uganda (Abstract 1134). Participants who were tested were stratified into active referral (CD4+ count > 100 cells/μL or enhanced referral (CD4+ count ≤ 100 cells/μL). Active referral cases were given a follow-up appointment within 3 months; enhanced referral cases were given an appointment within 2 weeks and initiated ART at the first clinic visit. The community population was 6300, and approximately 75% were tested. The adult HIV prevalence was 7.8%. Of 139 adults accepting referral to care, only 8 met criteria for enhanced referral. Of these, 6 (75%) linked to care successfully, all within 10 days, and initiated ART at their first clinic visit. Of the 131 participants who were classified as active referral, only 58% linked to care within 3 months; the lower the CD4+ cell count, the more likely the person would link to care. The authors concluded that the active-referral strategy was insufficient to achieve high levels of linkage to care.

Van Rooyen and colleagues presented a study on the efficacy of home-based HIV counseling in achieving linkage to care in KwaZulu-Natal, South Africa (Abstract 1135). The study enrolled 282 households with 743 adults,

91% of whom consented to HIV testing and point-of-care CD4+ cell count measurement. Only 33% of participants were male. Of all participants, 203 were newly identified as HIV-infected. By 3 months after HIV testing, 88% of those found to be seropositive had linked to care, and 11 of 13 eligible for ART were receiving it. The authors concluded that home-based testing was effective in reaching undiagnosed individuals with high CD4+ cell counts and achieved high rates of linkage to care.

The IeDEA cohort also presented a meta-analysis and review of linkage to care from diagnosis to ART initiation (Abstract 1143). The analysis used published data to determine the completion of 4 key steps in linkage to care: (1) HIV testing, (2) CD4+ cell testing with ART eligibility assessment, (3) ART eligibility, and (4) ART initiation. The data were drawn from 29 studies involving 148,912 ART-naive patients, and the researchers found that, of ART-eligible patients, only 62% initiated treatment (95% CI, 55.2%-70.7%). Mortality among those patients eligible for ART who did not initiate was 10.8% (95% CI, 4.6%-17.0%). Loss to follow-up occurred in 24.6% (95% CI, 10.8%-30.3%) of patients eligible for ART and 54.2% (95% CI, 42.8%-72.0%) of patients who were not eligible for ART. Based on these data, mortality and loss to follow-up prior to ART initiation are substantial barriers to ART coverage in RLS.

Geng and colleagues examined the issue of loss to follow-up in 4318 Ugandan patients entering HIV care with CD4+ cell counts greater than 350 cells/μL, making them ineligible for ART (Abstract 1151). A random sample of patients lost to follow-up was tracked in the community to determine vital status and clinical-care information. Over 2.5 years of follow-up, 1101 (35%) of patients initiated ART. Of the 858 patients (30%) lost to follow-up, 67 were tracked in the community, and outcomes were ascertained for 56 (84%) of them. Cumulative mortality at 2.5 years after last clinic visit for those lost to follow-up was 13.6% (95% CI, 5.5%-31.6%), and 56% of those thought to be lost to follow-up were found to be alive and in care at a new site. Although it is encouraging that many of those considered lost to follow-up were actually in care at a different site, the high mortality seen in this study corroborates the concern generated by the previously discussed presentations on linkage to care and follow-up.

Several groups explored retention in care and loss to follow-up after ART initiation. Elul and colleagues used data from 656 ICAP-sponsored treatment sites in sub-Saharan Africa to determine whether the proportion of nonretention in care at 6 and 12 months was changing over time and what factors were associated with nonretention trends (Abstract 86). The study aggregated program data from more than 316,762 patients older than 6 years in 5690 successive cohorts initiating ART quarterly from 2005 to 2010 were analyzed. Poisson regression with generalized estimating equations was used to show that there was no statistically significant change in nonretention at 6 or 12 months after ART initiation, with 27% to 29% of patients not being retained in care at 12 months throughout the observation period. When the investigators stratified nonretention at 12 months by country, they found statistically significant heterogeneity, with the lowest nonretention rates in Rwanda and the highest nonretention rates in Lesotho and Cote d'Ivoire. Nonretention was also examined by clinic maturity, and no statistically significant change in nonretention was found from 1 year to 5 years of clinic operation. Urban location, smaller clinic sizes, and lower CD4+ cell testing coverage at ART initiation were factors statistically significantly associated with nonretention at 12 months. One limitation to this analysis was that undocumented transfers of care were counted as nonretention events, but the authors noted that the lack of deterioration in retention rates over time during scale-up was encouraging.

Lamb and colleagues also used data from ICAP-sponsored sites to examine nonretention of loss to follow-up among 51,880 HIV-infected individuals 15 to 24 years of age (Abstract 1149). Loss to follow-up was defined as not returning to clinic for 6 months for those receiving ART and 12 months for those not receiving ART. Compared with adults from 25 to 54 years of age, HIV-infected youth had a relative risk of loss to follow-up of 1.30 (95% CI, 1.27-1.34) prior to ART initiation, and 1.64 (95% CI, 1.55-1.73) after ART initiation, after adjustment for country, facility type, location, point of HIV care entry, year of ART initiation, sex, pregnancy status, TB status, and CD4+ cell count at ART initiation. Risk factors for loss to follow-up before ART initiation included TB treatment (which was protective), earlier enrollment, and higher enrollment CD4+ cell count. Risk for loss to follow-up after ART initiation was associated with male sex, pregnancy, and CD4+ count at initiation either less than 100 cells/μL or 350 cells/μL or above. Youth between the ages of 15 years and 24 years appeared to be particularly at risk for loss to follow-up before and after ART initiation.

Finally, 2 investigator groups presented data on the efficacy of community-based adherence support, one in the form of one-on-one home visits (Abstract 1146) and the second using nonclinical adherence clubs run by health workers (Abstract 1150). Both studies showed increased rates of retention in care in the adherence support groups.

ART Outcomes in Children in RLS

The consequences of early versus deferred ART in children were examined in 3 presentations. Ananworanich and colleagues (Abstract 24) conducted a substudy on neurodevelopmental outcomes within PREDICT (Prospective Randomized Evaluation of DNA Screening in a Clinical Trial), which randomized 299 HIV-infected Thai and Cambodian children from 1 to 12 years of age with CD4+ percentage from 15% to 24% to either immediate ART or deferral of treatment until CD4+ percentage was less than 15%. The primary results of this study were presented by Puthanakit and colleagues at the 2011 International AIDS Society (IAS) conference; investigators

Table 2. Selected Presentations Regarding Linkage to Care and Retention in Care in Resource-Limited Settings

Study Description	Cohort Description	Key Findings
Abstract 1143 Meta-analysis of linkage to care from diagnosis to ART initiation	Sample: data published from 29 studies on 148,912 ART-naive patients Examined completion of 4 steps: 1) HIV testing, 2) CD4+ cell testing and ART eligibility assessment, 3)ART eligibility, and 4) ART initiation	• Of ART-eligible patients, 62% (95% CI, 55.2-70.7) initiated ART • Mortality among those patients eligible for ART who did not initiate was 10.8% (95% CI, 4.6-17.0) • Loss to follow-up occurred for: - 24.6% of those eligible for ART (95% CI, 10.8-30.3) - 54.2% of those not eligible for ART (95% CI, 42.8-72.0) *Conclusion:* Mortality and loss to follow-up prior to ART initiation are barriers to ART coverage in RLS, particularly for those who are not eligible for ART at diagnosis.
Abstract 1151 Examining loss to follow-up for those ineligible for ART at diagnosis in Uganda	Sample: 4,318 Ugandan patients entering care with CD4+ count >350 cells/μL Tracked a random sample of those who were lost to follow-up to determine status and clinical care information	• Over 2.5 years of follow-up, 1101 (35%) of patients with initial CD4+ count >350 cells/μL initiated ART • Cumulative mortality at 2.5 years for those lost to follow-up was 13.6% (95% CI, 5.5-31.6) • 56% of those lost to follow-up were found to be alive and in care at a new site *Conclusion:* More than half of those lost to follow-up were in care at a new clinical site, but those lost to follow-up had high mortality rates.
Abstract 86 Assessing changes over time in the proportion of nonretention in care at 6 and 12 months after ART initiation	Sample: Aggregate ICAP program data from over 316,762 patients >6 years of age in 5,690 successive cohorts initiating ART quarterly from 2005 to 2010 Poisson regression with generalized estimating equations used to assess changes in nonretention at 6 and 12 months	• No significant change in nonretention over time at 6 or 12 months • 27%-29% of patients were not retained at 12 months throughout the observation period • Urban location, smaller clinic sizes, and lower CD4+ cell testing coverage at ART initiation were associated with nonretention at 12 months *Conclusion:* Lack of deterioration in retention rates over time in a setting of rapid scale-up of treatment capacity across various sites is encouraging.
Abstract 1149 Determining loss to follow-up in 15-to 24-year-olds in RLS	Sample: 51,880 HIV+ individuals 15 to 24 years of age receiving care at an ICAP-sponsored site Determined predictors of loss to follow-up, defined as not returning to clinic for 6 months if receiving ART and 12 months if not	• HIV+ youth had a relative risk of loss to follow-up compared with adults 20-54 years of age: - Prior to ART initiation: 1.30 (95% CI, 1.27-1.34); associated with: tuberculosis treatment, which was protective; earlier enrollment; and higher enrollment CD4+ count - After ART initiation: 1.64 (95% CI, 1.55-1.73); associated with male sex, pregnancy, and CD4+ count at initiation either <100 or ≥350 cells/μL *Conclusion:* Youth aged 15 to 24 years appear to be particularly at risk for loss to follow-up both before and after ART initiation

ART indicates antiretroviral therapy; CI, confidence interval; ICAP, International Center for AIDS Care and Treatment Programs; HIV+, HIV seropositive; RLS, resource-limited settings

found AIDS-free survival rates of 98% in both arms at 144 weeks.[2] The neurodevelopmental substudy's primary objective was to test the hypothesis that neurodevelopmental outcomes at week 144 would be superior in the immediate ART group compared with the deferred ART group. Investigators enrolled 284 children from PREDICT using criteria described above. Control groups included 155 HIV-uninfected exposed children and 164 HIV-uninfected unexposed children. The researchers found that intelligence test scores did not differ between immediate and deferred ART groups, but both were statistically significantly lower than the HIV-uninfected control groups. Statistically significantly poorer outcomes were also seen in fine motor skills, memory scores, and behavioral scores for HIV-infected children than in HIV-uninfected controls. The authors compared their results to the CHER (Children with HIV Early Antiretroviral Therapy) study, which found that early ART was associated with improved neurodevelopmental outcomes for infants when it was initiated prior to 12 weeks of age. This suggests that a crucial window for early ART impact on neurocognitive development may have been missed in children who did not initiate ART until at least 12 months of age.

Wamalwa and colleagues presented

a different treatment strategy for infants, treatment interruption after early ART initiation. This approach was tested in Kenya in the OPHO3 (Optimizing Pediatric HIV-1 Therapy 03) Study (Abstract 27). The study included 42 infants who initiated ART aged 13 months or younger and who were on ART for more than 24 months, had CD4+ percentage of 25% or higher, and had normalized their growth. Participants were randomized in a nonblinded fashion to continued versus interrupted ART and monitored over 18 months for the endpoints of weight-for-height *z*-scores and serious adverse events. Treatment was reinitiated in the interruption arm if CD4+ percentage dropped below 25%, or participants experienced poor growth or a new opportunistic infection. There were no statistically significant differences between the 2 arms. Of the 21 children in the treatment interruption arm, 18 (86%) reinitiated ART; 14 of these reinitiated at 3 months because CD4+ percentage fell below 25%. The study's DSMB recommended that randomization be discontinued because too few participants remained in treatment interruption beyond 3 months, rather than for safety concerns. There were no statistically significant differences in growth or serious adverse events (SAEs) between the treatment interruption and continuation arms, but children who interrupted treatment had statistically significantly lower CD4+ percentages 15 months after randomization. Reinitiation of ART in the interruption group was associated with lower CD4+ percentage at randomization, with a median CD4+ percentage of 33% in the group reinitiating at or before 3 months, compared with a median CD4+ percentage of 39% in the group reinitiating at or after 6 months (P = .04). The investigators concluded that treatment interruption was not feasible for this patient population.

Cotton and colleagues presented the final data from 6 years of follow-up in the CHER trial (Abstract 28LB). The trial tested the utility of early, limited ART initiated at or before 12 weeks of age, followed by treatment interruption. The study randomized 375 children diagnosed with HIV infection before 12 weeks of age who had a CD4+ percentage of 25% or higher to 1 of 3 arms: (1) deferred ART; (2) early ART to 40 weeks followed by treatment interruption; or (3) early ART to 96 weeks. ART was (re)initiated in all 3 arms when CD4+ percentage dropped below 20% or participants experienced a clinical event. The primary study endpoint was time to failure of initial ART (lopinavir/r plus zidovudine and lamivudine); failure was defined as CDC category B or C clinical events, CD4+ percentage below 20%, or regimen-limiting toxicity. Within the first year of the study, a DSMB review found that the deferred ART arm had 16% mortality, and the other 2 arms had 4% in each. The deferred ART arm (arm 1) was stopped, 34 additional infants were enrolled in the study to maintain power, and early ART became standard of care for infants.[3] Baseline characteristics were not statistically significantly different among the 3 study arms, and 91% of participants completed the study. The HR for death or failure of the initial ART for the interruption after 96 weeks arm (arm 3) was 0.58 (95% CI, 0.35-0.96) compared with the ART deferred arm (arm 1), but was not statistically significantly different for the ART interruption after 40 weeks arm (arm 2). Comparing the 40- and 96-week arms (arms 2 and 3), there was no statistically significant difference in achievement of the primary endpoint. Only 7 participants switched to second-line ART in any of the arms. The authors concluded that early treatment initiation and continuation for 96 weeks versus 40 weeks prior to interruption resulted in similar ART exposure and longer subsequent treatment interruption, with no statistically significant differences seen in treatment failure or clinical events. Resistance data and other analyses from the 6-year follow-up data are forthcoming.

Lindsey and colleagues (Abstract 25) presented data from the P1060 trial, which compared the safety and efficacy of lopinavir/r-based regimens with nevirapine-based regimens for children 6 months to 3 years of age, 164 of whom had been exposed to sdNVP for PMTCT (exposed group) and 228 of whom had not been exposed to nevirapine (unexposed group). The primary outcome was permanent discontinuation of nevirapine or lopinavir/r or failure to achieve virologic control after 24 weeks on ART. The exposed group was statistically significantly younger, more likely to have HIV subtype C, and less likely to be breastfed than the unexposed group. The exposed group also had statistically significantly higher median CD4+ percentage, plasma HIV-1 RNA levels, and WHO weight *z*-score at baseline than the unexposed group. The DSMB for the study recommended early closure of accrual to the nevirapine-exposed group because the lopinavir/r arm showed superiority to the nevirapine arm for the primary endpoint. Cox proportional hazard models showed no statistically significant interaction between prior exposure to nevirapine and treatment effect. In the adjusted model, no statistically significant difference was seen between treatment response in the sdNVP-exposed and unexposed groups, but lower CD4+ percentage and higher plasma HIV-1 RNA levels predicted shorter time to the primary outcome. Interestingly, children in the nevirapine-based treatment arm had a statistically significantly greater increase in CD4+ percentage and weight-for-height *z*-score than those treated with lopinavir/r-based regimens. The dominant limitation of the analysis was that exposure to sdNVP was not randomized and the 2 cohorts had significant differences at entry. The investigators concluded that these data do not support the use of sdNVP as a reason to avoid nevirapine-based regimens for children between 6 months and 3 years of age, and speculated that the high HIV-1 plasma RNA levels at ART start and nevirapine initiation at half-dose may have led to the poor performance of nevirapine-based regimens in this investigation.

Prendergast placed the treatment strategy data reviewed above in context in a symposium on early ART initiation strategies in infants (Abstract

113). He explored data regarding 3 major strategic questions: (1) when to start ART, (2) what regimen to start, and (3) whether to switch or stop ART. A webcast of this symposium, which also discussed results from published trials relevant to these strategic questions, is available on the CROI web site (http://www.retroconference.org).

ART for Pregnant and Postpartum Women

An interesting addition to this year's CROI was a focus on the complexities of implementing ART in pregnant women in RLS and in non-RLS, highlighted in a themed discussion (Abstracts 1015-1019) and several other presentations (Abstracts 1005, 1006, 1020-1023). Anderson began the themed discussion with an emphasis on the current convergence between guidelines for ART in pregnancy in RLS and resource-rich settings, with the 2010 WHO guidelines for RLS recommending lifelong ART for those with CD4+ counts less than 350 cells/μL and encouraging triple ART prophylaxis for PMTCT if possible. The remaining discrepancy in RLS is predominantly because of the lack of virologic monitoring in such settings and the 2 options for discontinuation of ART in the setting of PMTCT: option A, 1 week postpartum, or option B, at delivery if formula feeding or 1 week after completion of breastfeeding. Recently, an "option B+" of lifelong ART for pregnant women regardless of disease status has been proposed, but concerns remain regarding the feasibility of ensuring lifelong adherence and retention in care, as well as the potential for toxicity or adverse pregnancy outcomes.[4] Further data on option B+ will be discussed in the PMTCT section in this review.

Fowler and colleagues explored a similar question in HIV-infected women in sub-Saharan Africa enrolled in the HPTN (HIV Prevention Trials Network) 046 study by examining disease progression in the first 12 months postpartum in women not meeting regional guidelines for ART initiation (Abstract 1015). Of 2025 HIV-infected women, 71% had a CD4+ count 350 cells/μL or higher at delivery. By 12 months postpartum, 26 (2%) of 1154 women with CD4+ counts 350 cells/μL or higher at delivery had dropped to CD4+ below 200 cells/μL. Of the 350 women with baseline CD4+ counts from 400 cells/μL to 549 cells/μL, 37% had CD4+ counts below 350 cells/μL at 12 months postpartum. Only 4.3% of all women progressed to AIDS based on immunologic or clinical criteria during the observation period. The authors concluded that, although progression to AIDS was relatively uncommon, the dramatic decreases in CD4+ cell counts over the 12 postpartum months could support an option B+ of initiating lifelong therapy for HIV-infected women during their first pregnancy.

Data on the difficulties of adherence during continuous ART in pregnancy and postpartum in RLS was presented by Kreitchmann and colleagues (Abstract 1016). The LILAC (Longitudinal Study in Latin American Countries) is a prospective cohort of HIV-infected women and their infants at 10 sites in Brazil, 1 in Argentina, and 2 in Peru. The study observes women from 22 weeks of pregnancy to 2.5 years after delivery and examines self-reported adherence to ART by 3-day recall. Of this cohort, 53% were using ART for PMTCT alone, and 47% met criteria for treatment. The study found a statistically significant decline in adherence rates from pre-delivery (mean percent adherence, 96.2%) to 6 months postpartum (mean percent adherence, 90.7%; $P < .005$), but no difference in adherence between those on ART for prophylaxis and those on ART for treatment. Multivariate models were developed to predict nonadherence at 3 time points: predelivery, 6 to 12 weeks postpartum, and 6 months postpartum. The final adjusted models showed that nonadherence was statistically significantly associated with current tobacco use predelivery (aOR, 2.9; 95% CI, 1.46-6.14), older age 6 to 12 weeks postpartum (aOR, 1.06/year; 95% CI, 1.00-1.12), and current alcohol use 6 months postpartum (aOR, 3.04; 95% CI, 1.34-6.90). The researchers concluded that adherence support is needed throughout the entire postpartum period, particularly in women using alcohol or tobacco.

Strategies for Laboratory Monitoring in RLS

Saag and colleagues presented data from a cluster randomized trial of HIV-infected individuals initiating ART in Zambia (Abstract 87). The trial compared routine plasma HIV-1 RNA testing (every 3 months for the first 6 months and every 6 months thereafter) with discretionary testing (available for anyone meeting criteria for clinical or immunologic failure at each visit and standard of care for the country). The primary endpoint of the study was all-cause mortality, with secondary endpoints of switch to second-line regimen and genotypic testing for resistance. The investigators highlighted that the plasma HIV-1 RNA level data from the discretionary testing arm was only 65% complete at the time of the presentation. The study found that overall mortality and virologic suppression rates were not statistically significantly different between the 2 arms. As anticipated, the switch to second-line regimen was higher (3.1 switches per 100 patient/years; HR for switch, 1.94; 95% CI, 1.22-3.10) in the routine monitoring arm than in the discretionary testing arm (1.6 per 100 patient/years) because the latter used changes in a patient's clinical or immunologic condition to trigger consideration of switch. Although the time to virologic failure was similar in both arms, the median time to switch was 168 days in the routine testing arm and 560 days in the discretionary testing arm ($P < .0001$). Limitations to this investigation included that it was underpowered to detect a mortality difference and unable to fully assess reasons for switch to second-line regimens. Additionally, constraints on formulary options for second- and third-line ART in Zambia may have influenced the willingness of providers to change regimens. The researchers concluded that routine virologic monitoring led to higher rates of change to second-line

regimens and less time on failing ART, and highlighted that it was feasible to successfully embed this operational research project into routine care at PEPFAR clinics. HIV genotype analyses, cost effectiveness analyses, and detailed examinations of adherence interventions are forthcoming.

Egger and colleagues addressed the problem of lack of virologic monitoring in RLS by using data from the IeDEA cohort to develop a chart of risk for virologic failure based on CD4+ cell count over time (Abstract 634). The researchers incorporated data from all IeDEA cohorts in South Africa where routine virologic monitoring was available, and included patients with baseline CD4+ cell count, age, sex, and more than 1 plasma HIV-1 RNA level and CD4+ cell count cell count at least 6 months after ART initiation. CD4+ cell count trajectories were analyzed in patients with virologic suppression using mixed models and fractional polynomials, stratified by pre-ART CD4+ cell count. The converse analysis, predicting virologic failure for the same CD4+ categories, was conducted using Poisson models. These data were combined to create overlapping charts of CD4+ cell count trajectories over time and risk of virologic failure, with separate charts for men and women in each of the 4 baseline CD4+ cell count strata. These risk charts were then validated with data from the TREAT Asia (Therapeutics Research, Education, and AIDS Training in Asia) HIV Observational Database (TAHOD). CD4+ cell count trajectories with suppressed viral replication were somewhat more favorable in women than in men, and the charts were most accurate in predicting virologic failure in patients initiating ART at lower CD4+ cell counts. The investigators suggested that these risk charts could be used to support decision-making with respect to switching to second-line therapy in programs without access to plasma HIV-1 RNA level monitoring.

A highlight of the laboratory monitoring in RLS discussion was Sherman's presentation on the challenges of HIV diagnosis in infants in RLS (Abstract 112). In 2004, when ART became available for the first time for infants in RLS, the WHO guidelines recommended a single PCR test at 6 weeks of age, with the timepoint chosen because it coincided with scheduled immunization visits. From 2004 to 2010, there was an impressive scale-up of PCR diagnostic testing in South Africa. However, access to this technology remains limited, not all HIV-exposed infants are tested, and some infants who test positive for early infection with HIV are not accessing ART. Despite these caveats, the increased availability of PCR testing for infant diagnosis has enabled South Africa to use PCR test results to monitor the success of PMTCT programs by region. Using estimated numbers of infants who should be tested by area, early infant diagnosis coverage and early transmission rates among those infants who were tested can be determined. Sherman cited this as a success in allowing a transition from national to local control and accountability for PMTCT efforts.

Current testing available for early infant diagnosis is increasingly automated, leading to decreased technologist time, increased sensitivity, and improved specificity, all of which may enable earlier diagnosis, even in the setting of ART for PMTCT. Point-of-care testing platforms for early infant diagnosis are also being evaluated, but none have been launched. Sherman discussed the underutilization of rapid HIV antibody detection assays, which can identify HIV-exposed infants and can exclude infection in older HIV-exposed infants. For children younger than 18 months, rapid test performance is less uniform than in adults and tests must be chosen based on specific clinical application, whether that be testing for exposure or seroreversion because of the passive transfer of maternal antibody to the infant.

Sherman presented unpublished data from her own laboratory of infant diagnosis algorithm testing in 838 mother/infant pairs using 2 plasma HIV-1 RNA measurement assays, 1 at birth and the other 6 weeks later. Of those, tests showed 43 infants were infected: 29 in utero (positive PCR for HIV infection at birth), 9 intrapartum (negative PCR at birth and positive PCR at 6 weeks), and 5 postpartum (positive HIV test after 6 months of age). At the 6-week PCR test for this same cohort, 18 of the intrauterine infections and 8 intrapartum infections were detected. Thus, 76% of all early infections within the cohort would have been detected at birth. Using the standard of care 6-week test meant that 11 (32%) of the infants diagnosed retrospectively with early HIV infection were lost to follow-up. Sherman also discussed the mounting evidence that prolonged PMTCT prophylaxis may delay infant diagnosis, including her own laboratory's data, which showed that a single dose of nevirapine and zidovudine led to 4 of 18 HIV-infected infants having negative plasma HIV-1 RNA levels. Sherman stated that research priorities should focus on data collection directed at a revision to the testing algorithm, perhaps with at-birth testing using a point-of-care test.

PMTCT

Two oral presentations on PMTCT efforts placed particular emphasis on the advent of the 2010 WHO Guidelines and the Joint United Nations Programme on HIV/AIDS (UNAIDS) Global Plan to reduce mother-to-child transmission (MTCT) to less than 5% of the current rate by 2015.

As part of the CROI Workshops for New Investigators and Trainees, Flynn reviewed the successes in, barriers to, and consequences of PMTCT (Abstract 5). She reflected on the evolution of the understanding of risk of HIV transmission to infants in utero, during labor and delivery, and during the breastfeeding period, and she reviewed how such understanding has led to effective new PMTCT strategies. She highlighted exciting innovations in the field and areas of growth such as promising immune therapies, enhanced testing initiatives, programs to optimize engagement in care, outcomes data on children with HIV or ART exposure, and novel point-of-care technology.

Mbori-Ngacha echoed many of these themes in her plenary session that opened the third day of the conference,

dedicated to the elimination of MTCT of HIV (Abstract 75). She provided an overview of PMTCT strategies with emphasis on the scope of MTCT, scientific advances, and opportunities for global elimination, describing the Global Plan for the elimination of MTCT. Staggering statistics from 2010 regarding ongoing MTCT of HIV were cited, with 390,000 children infected with HIV globally and 92% of these infections having occurred in sub-Saharan Africa. The Global Plan, which focuses on 22 countries (located primarily in sub-Saharan Africa) that bear 91% of the global burden of MTCT of HIV, has 2 main targets: to reduce MTCT to less than 5% in HIV-infected breastfeeding mothers and to reduce the number of AIDS-related maternal deaths by 50% by 2015. UNAIDS is proposing a multi-pronged approach for PMTCT in RLS, including prevention of HIV in young women, prevention of unintended pregnancies in HIV-infected women, prevention of transmission from HIV-infected women their infants, and support for infected mothers and their families.

Mbori-Ngacha reviewed the benefits and drawbacks of options for PMTCT at higher CD4+ cell counts. Although option A (maternal zidovudine and 6 weeks of infant prophylaxis with nevirapine or zidovudine) is associated with lower rates of infant adverse events, costs less, and requires less monitoring, this option requires a known CD4+ cell count, may be more complex to deliver, and is associated with a substantial risk of NNRTI resistance. On the other hand, option B (triple drug ART prophylaxis) offers uniformity in treatment for pregnant women, prevents transmission to uninfected partners, and may provide additional health benefits to the mother during pregnancy, but there are concerns about increased cost, more necessary safety monitoring, and more adverse effect than option A, as well as the potential for drug resistance.

Application of PMTCT Guidelines

Rundare and colleagues presented data on the effect of the uptake of the South African National Department of Health PMTCT Guidelines in South Africa (Abstract 1003). Investigators compared outcomes in women observed between June 2009 and June 2011. A cohort of 1995 mother-infant pairs was included, with 1147 mothers attending prior to these guidelines being released and 848 attending after the guidelines' release. Transmission rate prior to the guideline change was 3.4% (95% CI, 2.6%-5.0%) and dropped to 1.5% (95% CI, 0.9%-2.9%; $P = .2$) after the implementation of the guidelines. The authors concluded that the South African guidelines were effective and associated with decreased MTCT of HIV at 6 weeks.

French and colleagues reported on rates of repeat pregnancies among HIV-infected women, stratified by immunologic status and virologic outcomes for women not on ART at conception (Abstract 1019). Based on analysis from the United Kingdom/Ireland National Study of HIV in Pregnancy and Childhood, 1177 second pregnancies were identified from 2000 to 2010 in women not on ART at conception. At the time of the second pregnancy, the median CD4+ count was 392 cells/μL, with 40% of women having CD4+ counts less than 350 cells/μL and 24% with a CD4+ count less than 200 cells/μL. Investigators found that two-fifths of women had an immunologic indication for ART at the start of their second pregnancy. Nearly half of these women had had CD4+ counts of 350 cells/μL or higher at their first pregnancy. There were high rates of antenatal ART (97%); however, most started during or after the second trimester and 27% of women not on ART at conception had detectable plasma HIV RNA levels at delivery. The risk of a detectable plasma HIV RNA levels at delivery was associated with timing of ART initiation. The authors urged consideration of lifelong ART at first pregnancy for all women (option B+).

Availability of PMTCT Services

The effort to reduce MTCT rates to less than 5% of the current number by 2015 is hindered by many factors: inadequate coverage of PTMCT treatment in many settings, cost considerations, and political commitment.

Chi presented on field effectiveness of universal ART for PMTCT in rural Zambia (Abstract 23LB). In a prospective cohort study, 9 rural facilities in Zambia were analyzed. Four sites offered universal ART regardless of CD4+ cell count, and 5 sites provided standard-of-care treatment: short-course zidovudine starting at 28 weeks of pregnancy and through the intrapartum period and sdNVP intrapartum with a zidovudine/lamivudine tail. Infants received sdNVP followed by a week of zidovudine. From April 2009 to December 2010, 284 women were enrolled; 143 received universal ART and 141 received short-course therapy. At 12-month follow-up, rates of HIV infection or death were higher in the short-course therapy arm (19.8% compared with 5.8% in the universal ART arm; RR, 3.44; 95% CI, 1.56-7.59). The rate of loss to follow-up was higher in the universal ART arm. The authors offered this as evidence in support of 2010 WHO guidelines recommending option B over option A and urged tailored interventions to improve adherence and retention in care for pregnant and postpartum women.

Assessments of PMTCT program outcomes in the United States were presented by Taylor and colleagues (Abstract 1000). Using published national estimates of rates of perinatal ART exposure, cases prevented, and infected infants in the era of ART prophylaxis, the authors reported 124,280 HIV-exposed infants, 25,659 cases of MTCT prevented, and 102,543 infants exposed to ART from 1994 to 2010. Nationally, there were 183 and 151 cases of MTCT in 2006 and 2009, respectively. The authors pointed out that the incidence of perinatal HIV infection continues to exceed the goal of 1 infection per 100,000 live births.

Diagnostics and MTCT

Given that women with low CD4+ cell counts are more likely to transmit HIV to their infants perinatally and have high mortality rates, identification and treatment of pregnant women with

low CD4+ counts is a top priority. Delays in CD4+ cell count testing can hinder care. Furthermore, awareness of a low CD4+ cell count can motivate women to engage more fully in care. Mnyani and colleagues presented data on the role of point-of-care CD4+ testing in a PMTCT setting (Abstract 1007). The investigators compared the performance of the Pima™ analyzer, a point-of-care CD4+ count machine, with laboratory-based CD4+ cell testing in a high-volume PMTCT clinic in South Africa. Parallel CD4+ cell testing was performed on 232 consecutive HIV-infected pregnant women. The median Pima™ CD4+ count was 350 cells/μL (IQR, 231-488) and for the laboratory-based assay the median count was 362 cells/μL (IQR, 246-522). The mean difference in laboratory minus Pima™ CD4+ count was 13.9 cells/μL (95% CI, 5.1-22.7) and no difference was found in the level of agreement related to participant age or time of gestation.

Acute HIV Infection in Pregnancy and Postpartum

Several studies have suggested an increased risk of HIV transmission to women during pregnancy, higher rates of MTCT for women with primary HIV during the perinatal period than those with chronic HIV, and high rates of unintended pregnancies among HIV-infected women in RLS. Marum and colleagues presented modeling data from Zambia suggesting that incident acute infection during pregnancy and lactation among women testing HIV-seronegative early on in pregnancy results in more infant infections in Zambia than regimen failure and lack of coverage combined, and urged joint couples testing and treatment of HIV-serodiscordant couples to advance prevention of MTCT (Abstract 1001).

Family Planning

Availability of effective contraception for HIV-infected women was an important theme at the conference. Sutton and colleagues reported on unplanned pregnancies among women in care for HIV infection and followed up as part of the MMP in sites in the United States and Puerto Rico (Abstract 1044). Of 1407 women in the study, 370 (26.3%) reported 1 or more pregnancy after HIV diagnosis, and 316 of these pregnancies (85.6%) were unplanned. The authors called for strengthened contraception awareness and access for women. Heffron and colleagues reported no association between HIV disease progression and hormonal contraceptive use in a prospective cohort of 2236 HIV-infected women in Africa ($P = .03$) (Abstract 21).

Novel Immune Methods for Treating Women During Pregnancy

Although ART has been the mainstay of PMTCT during all periods of potential perinatal transmission, novel methods that incorporate vaccines or neutralizing antibodies may be part of future regimens. Session 183 was dedicated to research on the role of neutralizing antibodies during MTCT. Mabuka and colleagues found no evidence for an association between HIV neutralizing antibody levels in breast milk and infant HIV infection in a small study of 10 women who transmitted to their children during breastfeeding compared with 7 women who did not (Abstract 1035). Omenda and colleagues found that maternal neutralizing antibodies correlated with infant passive antibodies but not with the risk of MTCT in a study of 22 transmitting and 51 nontransmitting mother/infant pairs (Abstract 1036). These early studies, among others, provide contextual background for the potential use of broadly cross-active neutralizing antibodies as passive therapy to reduce MTCT rate.

Breastfeeding

Although avoidance of breastfeeding where safe formula is attainable may reduce MTCT, in some RLS this benefit has been neutralized by higher infant mortality rates as the result of the absence of nutritional and immunologic benefits provided by breast milk.[5] Such findings have resulted in a multitude of studies to identify methods to lower the risk of transmission during the breastfeeding period, including studies aimed at understanding risks associated with patterns of breastfeeding and weaning and options for either maternal or infant ART to prevent transmission. Postnatal MTCT rates are comparably low across studies that have included maternal triple ART during the breastfeeding period. Additionally, there have been important studies showing that infant prophylaxis is also effective during the breastfeeding period. The BAN (Breastfeeding, Antiretrovirals and Nutrition) Study of infant nevirapine or maternal triple ART in nursing mothers found that both active strategies statistically significantly reduced transmission compared with no intervention. However, due to inadequate statistical power, it remains unclear which active method is more effective.

Adherence and Retention in Care for PMTCT

The ambitious goal of a 90% reduction in MTCT will require careful attention to ART adherence and retention in care.

Myer and colleagues presented 12-month outcome data on loss to follow-up and mortality among pregnant and nonpregnant women initiating ART from IeDEA Southern Africa (Abstract 22). The analysis included 29,653 treatment-naive, HIV-infected women aged 16 years to 56 years with CD4+ counts below 200 cells/μL who initiated ART from 2002 to 2009. Of these, 1956 were pregnant at the time of ART initiation. Pregnant women were younger at the time of ART initiation (29 years vs 33 years old in nonpregnant women) and had higher CD4+ counts (median, CD4+ 145 cells/μL, compared with 96 cells/μL in non-pregnant women). After 12 months on ART, 3% of women who had been pregnant at the time of ART initiation had died, compared with 9% of women who were not pregnant at the time of ART initiation. Loss to follow-up was higher in those who had started on ART when pregnant, with 19% of pregnant women lost to follow-up, compared with 11% of nonpregnant

women lost to follow-up. The HR for loss to follow-up remained elevated even with adjustment for pregnancy, age, baseline CD4+ cell count, year of ART initiation, and participating site (adjusted HR, 1.7; 95% CI, 1.49-1.94). The authors also cited an urgent need for interventions to promote retention of women initiating ART in pregnancy to achieve optimal maternal and child health outcomes, especially in light of aggressive plans to scale up PMTCT programs.

Rawizza and colleagues evaluated rates of loss to follow-up within the PMTCT Care Cascade in a large ART program in Nigeria (Abstract 1017). A retrospective analysis of 33 clinical PMTCT sites was performed between 2004 and 2011. Among 19,303 women who entered care during the antenatal period, 10,078 (52%) completed the entire cascade of services (antenatal care, delivery, and infant follow-up). Among 22,180 women entering care at any point along the PMTCT cascade, only 2933 (13%) of their infants were retained in follow-up care through 18 months. The greatest loss to follow-up occurred after delivery and before infant follow-up with unknown outcomes for infants of 45% of mothers who had received any antenatal care. The authors urged strategies to improve retention in care, especially during the transition from pregnancy to pediatric care.

Outcomes in Children Exposed to HIV but Uninfected

Although outcomes of HIV infection in children have improved dramatically with combination ART, exposure to HIV or ART is also associated with toxic effects, including premature birth, anemia, and impaired growth. Session 34 was dedicated to recognition of the risks of exposure to HIV and ART in infants and children (Abstracts 110-113). Blanche focused on the consequences of zidovudine exposure for PMTCT, acknowledging the extraordinary efficacy of ART for PMTCT; he also described morbidity related to in utero exposure to ART, including premature birth and a spectrum of findings around the effects of zidovudine (Abstract 110). He presented unpublished data on the possible genotoxic effect of zidovudine on hematopoietic stem cells from a comparison of the genotypic profiles of stem cells derived from cord blood of HIV-infected versus -uninfected mothers. There were statistically significant differences between HIV-infected and -uninfected subjects' cord blood stem cells in upregulation and downregulation of various genes involved in cell cycle and DNA repair. There were higher levels of aneuploid cells in HIV-infected mothers as well. The author urged ongoing efforts to identify the safest PMTCT approaches through observational cohorts, randomized trials, and new biologic tools.

Filteau presented an update on the current understanding of the vulnerabilities of HIV-exposed, -uninfected children (Abstract 111), acknowledging several health conditions seen in HIV-uninfected children exposed to HIV or ART. Data have revealed high rates of mortality and hospitalization and poor growth in this group, and raised questions about possible cognitive problems and risks of chronic diseases. The author suggested that HIV exposure, maternal HIV infection and disease severity, ART exposure, increased infections, and decreased breast feeding are responsible for these differences in risks and urged more research to mitigate these differences and maximize community interventions in support of vulnerable HIV-exposed but -uninfected children.

Warsawski and colleagues presented data on infant mortality among children not infected perinatally with HIV during the potent ART era (Abstract 1032). Investigators analyzed infant mortality rates for uninfected infants born between 1997 and 2009 to HIV-infected women in the ANRS French Perinatal Cohort and found higher rates of infant mortality in premature infants compared with term infants (27.0/1000 live births vs 3.5/1000 live births; $P < .001$). Compared with the general population, premature births were more common among HIV-infected women (14.8% in the HIV-infected women vs 6.3% in general population). The authors concluded that the high premature birth rate was responsible for the higher infant mortality rate in children born to HIV-infected women and questioned whether ART could be playing a role.

Cade and colleagues suggested a possible relationship between in utero exposure to ART and lower diastolic function and left ventricular mass index in HIV-uninfected children (Abstract 1034). Investigators studied echocardiographic and other cardiac parameters in 30 HIV-uninfected children born to HIV-infected women with a history of in utero ART exposure and compared the findings with 30 HIV-uninfected children without exposure to ART or HIV. Left ventricular mass index was statistically significantly lower in the ART-exposed children (61 ± 9 g/m^2 vs 66 ± 12 g/m^2 in the control children; $P < .04$) and early diastolic annular velocity was also lower (14.9 ± 2.2 cm/s vs 16.4 ± 2.5 cm/s, respectively; $P < .02$). The authors suggested that these findings could be residual effects of ART exposure, but maternal HIV infection and in utero HIV exposure may also play a role.

ART Resistance

Resistance in RLS

Lack of HIV RNA level monitoring in RLS has been associated with prolonged initial treatment failure and accumulation of drug resistance mutations. The WHO guidelines recommend NNRTI-based ART for initial therapy and a PI/r-based regimen as second-line therapy. Hamers presented patterns of HIV-1 drug resistance in 2 cohorts followed up at 13 sites in sub-Saharan Africa. The first cohort enrolled ART-naive patients and observed them for evidence of failure of initial ART at 12 months and the second cohort included individuals on second-line ART (Abstract 104). More than 2000 ART-naive patients who started NNRTI-based ART were in care at 12 months, at which point 90% of patients had an HIV RNA level of less than 400 copies/mL. Of 166 patients with HIV RNA levels above 1000

copies/mL whose drug resistance test results were available, 100 carried 1 or more drug resistance mutations and 96% of detected drug resistance mutations were not evident at baseline. The most common mutations identified included M184V or an NNRTI resistance-conferring mutation (such as K103N and Y181C). In the second cohort analysis, 243 patients switched empirically to second-line ART, of whom 104 were predicted to receive a fully active second-line regimen and 128 were predicted to receive only a partially active second-line regimen based on the resistance profile at the switch. Those who received a partially active regimen were more likely to have AIDS at switch and to have had a longer median duration of initial ART. Most second-line regimens included lopinavir/r as the PI/r. However, the nRTI selection varied. At 12 months, 173 of 201 (86%) patients in care on a second-line regimen had an HIV RNA level of less than 400 copies/mL. Interestingly, an adjusted multivariate logistic regression analysis model predicting virologic failure of second-line regimens at 12 months did not find an association between expected activity of the second-line regimen and virologic failure (OR, 0.80; 95% CI, 0.33-1.91; $P = .610$).

Patterns of drug resistance among patients on PEPFAR nRTI/NNRTI regimens in Nigeria were covered in Abstract 738. Ndembi and colleagues presented data on patients who experienced virologic treatment failures on an initial NNRTI-based regimen and received HIV-1 RNA testing in 2010. G and CRF02_AG were the most common subtypes. Of 219 patients with virologic failure, 21% had no evidence of drug resistance; 73.1% harbored nRTI resistance; 68.9% had an M184I/V mutation; 13.2% had at least 3 thymidine analogue nRTI mutations; and 17% had K65R, 6 of whom were on tenofovir. NNRTI resistance was found in 74% of samples. Of these, 36.1% were Y181C mutations and 31.0% were K103N mutations. Two or more etravirine-associated mutations were seen in 53% of patients and 2.7% of patients had major PI mutations.[6] The authors recommended genotypic monitoring as part of routine care.

Estimated prevalence of genotypic drug resistance in ART-naive persons in sub-Saharan Africa and Southeast Asia was presented in Abstract 739. Rhee and colleagues analyzed epidemiologic and sequence data from studies of targeted ART-naive populations of at least 25 people in sub-Saharan Africa and Southeast Asia. Drug resistance mutation rates were calculated and compared. In 72 sub-Saharan Africa studies, the median drug resistance mutation prevalence was 3.3% (IQR, 1.6-5.7). In 31 Southeast Asia studies, the median drug resistance mutation prevalence was 2.4% (IQR, 1.3-6.1). Of the combined Sub-Saharan Africa and Southeast Asia populations (representing 8793 persons from 103 studies), 15 drug resistance mutations were identified in at least 0.1% of the population. These 15 mutations accounted for 67% of all drug resistance mutations, which included 8 nRTI resistance mutations (M41L, M184V, K219Q, D67N, T215S, K219N, L210W, V75M); 4 NNRTI resistance mutations (K103N, Y181C, G190A, K101E); and 3 PI resistance mutations (M46L, M46I, L90M). The nRTI resistance mutation V75M and the PI resistance mutation M46I were more common in Southeast Asia, due to increased frequency in HIV subtype CRF01_AE. In both sub-Saharan Africa and Southeast Asia, the prevalence of NNRTI resistance mutations increased over time (OR, 1.003; $P < .001$). In sub-Saharan Africa, but not in Southeast Asia, the prevalence of nRTI resistance mutations increased over time.

Kiertiburanakul and colleagues presented comparisons of major HIV drug resistance mutations in recent and chronic HIV infection among Asian patients (Abstract 740). The investigators included ART-naive patients enrolled in the TREAT Asia studies from 2007 to 2010. There were 458 patients with recent infection and 1340 patients with chronic infection included in the analysis. Patients with recent infection were younger (median age 23 years vs 36 years), more likely to be men (91.9% vs 65.9%), and less likely to report heterosexual HIV exposure (13.1% vs 72.2%) than patients with chronic infection, and also had lower HIV RNA levels (median 4.7 $\log_{10}$ copies/mL vs 5.0 $\log_{10}$ copies/mL) and higher CD4+ count (median 349 cells/μL vs 104 cells/μL). The crude prevalence of patients with at least 1 resistance-associated mutation to any drug class in both cohorts was 4.6%. There was a greater frequency of resistance-associated mutations PIs among those with recent infection than among those with chronic infection (3.9% vs 1.0%, respectively; $P < .001$). In multivariate logistic regression analyses of the chronic infection group, those with heterosexual contact as a risk factor for HIV were less likely to have resistance-associated mutations (OR, 0.34; 95% CI, 0.20-0.59; $P < .001$).

Boltz showed that the risk of virologic failure associated with low-frequency nevirapine-resistant variants of HIV in women initiating nevirapine-containing ART varies depending on the history of exposure to sdNVP in OCTANE/ACTG 5208 (Abstract 105). Previously, investigators reported that nevirapine-resistant variants at frequencies greater than 1% in pretherapy samples were statistically significantly associated with failure of initial ART with nevirapine-based, but not lopinavir/r-based, regimens among women with prior exposure to sdNVP in OCTANE/ACTG 5208 Trial 1. In contrast, OCTANE Trial 2 studied women without prior exposure to sdNVP and found no difference in primary study endpoints (virologic failure or death) with nevirapine-based ART. The authors hypothesized that this was due to the absence of low-frequency nevirapine-resistant variants in women without prior sdNVP exposure. To test this hypothesis, pretherapy samples from both trials were tested for nevirapine-resistant variants by allele-specific PCR (ASP) and the results were related to study endpoints of virologic failure and death. Surprisingly, nevirapine-resistant variants were commonly detected in baseline samples from the non-sdNVP-exposed women in Trial 2 (38 of 211 samples, 18%). Although the frequency of mutation detection was less than in Trial 1 (51 of 114 samples, or 45%; $P < .001$), it was more

frequent than expected. Study endpoints in women without ASP-detected nevirapine resistance had similar study endpoint rates in both trials: 31 of 173 (18%) in Trial 2, versus 9 of 63 (14%) in Trial 1 ($P = .56$). Also surprising was that the nevirapine-resistant variants detected by ASP were not associated with risk of a primary study endpoint in Trial 2 ($P = .88$), whereas in Trial 1 they were associated with increased risk ($P = .001$). The authors hypothesized that this apparent paradox may be explained by the greater size of resistant virus populations after sdNVP exposure, which may increase the likelihood of additional drug resistance associated mutations accumulating on the same viral genome. It could also secondary to the specificity of this assay and the threshold with which it designated nevirapine resistance.

Among HIV-infected infants with a history of nevirapine exposure followed by subsequent treatment with nevirapine-containing ART, low-frequency resistance mutations present at baseline were commonly selected during virologic failure according to Lehman and colleagues (Abstract 721). The authors compared rates of low-frequency mutations by ultra-deep sequencing (UDS) with outcomes in 20 nevirapine-exposed infants. UDS revealed 1 or more resistance-associated mutations in 12 of 20 infants (60%). The risk of virologic failure was increased in infants with baseline mutations (HR, 2.21; 95% CI, 1.41-3.48; $P = .001$). All 7 infants who experienced virologic failure had resistance identified by population sequencing, and of these 7, 6 had multi-class resistance. Mutations present at frequencies of less than 1% did not grow out during virologic failure; however, 3 of 4 mutations present at levels between 3.8% and 50% before ART were detectable at virologic failure with population sequencing. Authors suggested that minority variants present prior to ART in infants with nevirapine exposure may predict virologic failure.

ART Resistance in Children

Session 168 was dedicated to HIV drug resistance and tropism after treatment failure in children (Abstracts 988-991). Westley and colleagues explored the accurate detection of virologic failure and drug resistance in children based on immunologic follow-up without routine HIV RNA monitoring, per the 2010 WHO guidelines (Abstract 988). The investigators studied drug resistance patterns in children under 15 years of age who had been on at least 6 months of initial ART (nevirapine-based in most cases) and compared rates of immunologic failure with virologic and genotypic results. Of 51 children with evidence of virologic failure on initial ART, genotypic analysis revealed drug resistance in 98% of samples. Only 1 case would have been accurately categorized as treatment failure by immunologic criteria. The authors advocated for availability of affordable and routine HIV RNA monitoring for HIV-infected children.

Chohan and colleagues reported rates of the emergence of nevirapine resistance in 22 HIV-1-infected infants initiating nevirapine-based ART in Nairobi, Kenya. All 22 were less than 5 months of age, had not been exposed to nevirapine, and were confirmed to have no preexisting nevirapine resistance mutations (Abstract 989). Resistance emerged in 7 infants after 12 months of nevirapine-based ART (32%) and none of the infants achieved viral suppression. All 7 infants had a nevirapine-associated mutation and an additional M184V nRTI mutation. Based on these high rates of nevirapine resistance, the authors urged consideration of PI/r-based regimens as an alternative to nevirapine.

The InSTI class represents a promising new class of antiretroviral drugs under study for use in children. Nelson and colleagues presented an analysis on the presence of InSTI resistance mutations in InSTI-naive children (Abstract 990). Primers were designed for reverse transcription, nested PCR, and population sequencing using the group M consensus sequence. Primers were validated using adult samples with known mutations. A total of 86 unique samples were sequenced from individual children enrolled in the PACTG (Pediatric AIDS Clinical Trials Group) 390 and IMPAACT (International Maternal Pediatric Adolescent AIDS Clinical Trials) P1066. In all, 76 children had HIV subtype B, 6 had subtype C, and 4 had a circulating recombinant form of HIV-1 (CRF02_AG). Though 3 children had minor resistance mutations, none of the children had any major InSTI resistance mutations. The investigators concluded that baseline InSTI resistance is no more likely in children than adults.

New Drug Resistance Testing Assays

Jabara and colleagues presented a novel technique to accurately resolve low-abundance drug-resistant variants in therapy-naive individuals. Traditional deep sequencing requires a large amount of input material and clinical samples typically possess a limited number of viral templates. PCR is a necessary first step to generate the necessary input material before deep sequencing, and it can introduce a substantial degree of bias. Examples of bias found in standard deep sequencing include increased artifactual diversity, disruption of true linkage, skewed allelic frequencies, failure to control for resampled sequences, and high rates of sequencing errors. In the presented technique, each individual complimentary DNA (cDNA) molecule is tagged with 2 codes: a sequence unique to the sample that serves as a barcode for the sample, and a Primer ID, a string of degenerate nucleotides unique to each cDNA molecule. The Primer ID then serves as a unique tag to track the cDNA origin of all amplified templates. Sequences originating from the same cDNA molecule can be pooled to generate a consensus sequence, which should remove errors present in the individual amplified sequences.[7] The researchers applied this technique to HIV-1 protease and HCV nonstructural protein 3 (NS3) (Abstract 99). In the presented technique, the viral RNA was tagged with the Primer ID during cDNA synthesis, which was followed by the generation of consensus sequences. Examples of use of the Primer ID for deep sequencing of

the HIV-1 protease and the HCV NS3 were presented. The technique demonstrated accurate sampling and deep sequencing of viral populations, detection of resistance mutations with a frequency of less than < 0.5%, and ability to correct for PCR biases, sequencing error, and PCR resampling.

Phylogenetic Modeling and Population Analysis

Several presentations in session 100 were dedicated to phylogenetic and population analysis techniques. Using phylodynamic methods, Volz and colleagues performed an epidemiologic analysis of *pol* sequences that had previously been collected for surveillance of drug resistance in southeastern Michigan (Abstract 534). The authors analyzed 1252 HIV-1 subtype B *pol* sequences collected for drug resistance testing among MSM. The sequences represented 70% of infections diagnosed in southeastern Michigan between 2004 and 2010. Traditional data sources used in tandem with the genetic data included time series of all diagnoses from 1985 to 2010 and demographic covariates for each patient. Phylogenetic analyses showed a high degree of transmission clustering within age (*r*, 16.1%) and race (*r*, 38.2%) categories; early/acute infections were highly clustered within the phylogeny (*r*, 14.2%). The analysis demonstrated an increased incidence of infection among black teenaged MSM since 2007. This increase appears to be driven by transmission within this demographic group rather than spillover from older individuals.

Predictors of Resistance

Zheng and colleagues explored possible associations between pretreatment CD4+ cell count and pretreatment HIV-1 RNA levels and the emergence of drug resistance at virologic failure on efavirenz plus nRTIs (Abstract 705). The analysis included participants in 3 ACTG trials who initiated efavirenz and nRTIs and had genotypic data from baseline and at virologic failure. Drug resistance was defined by new mutations not present at baseline. Rates of drug resistance across CD4+ cell count strata were assessed and multivariable logistic regression models were used to adjust for baseline demographics. There were 196 patients included in the analysis and the median time to study-defined virologic failure was 33 weeks. At virologic failure, 113 patients (58%) had new NNRTI resistance mutations. No statistically significant trend across CD4+ cell count strata in the proportions with new NNRTI mutations was observed (P = .43). Similar results were obtained adjusting for pre-ART HIV-1 RNA (P = .21), prior nonadherence (P = .45), and baseline demographics (P > .10). No statistically significant trend was observed across pre-ART HIV-1 RNA levels strata (P = .29). Analyses of nRTI resistance gave similar results. A multivariable model adjusting for baseline covariates and prior nonadherence showed younger age was associated with new NNRTI resistance (< 30 years vs ≥ 45 years; OR, 4.1; 95% CI, 1.4-12.3, P = .037).

Transmission of Low-Frequency Drug Resistance

Lipscomb and colleagues reported transmission of numerous low-frequency drug–resistant HIV variants detected during acute HIV infection (Abstract 571). The study employed sensitive real-time PCR testing targeted to detect resistance mutations during acute HIV infection in an effort to understand the range of virus expression immediately following transmission. Longitudinal plasma samples were collected from 13 seroconverters every 5 to 7 days and screened for evidence of thymidine analogue nRTI-resistant mutations M41L, K70R, and K65R. This screening was followed by further clonal analysis in samples that screened positive for mutations. Transmitted resistance mutations were found in 3 (23%) of the people with acute infections: 1 with both K65R and K70R mutations, 1 with K65R, and 1 with K70R. In the individual with K65R and K70R mutations, sequencing verified K65R in 16.7% of the clones 5 days prior to seroconversion. The K65R variant coexisted unlinked with 5 distinct thymidine analogue nRTI-resistant variants, clones with M184V/I, and 5 NNRTI-resistant variants. Wild-type virus comprised 38% of the swarm. By 8 days postseroconversion, only 1 mutation was detected, at 2%. Analysis of *env* at 5 days preseroconversion in the dual-mutation infection revealed few clones with 2 or fewer polymorphisms relative to the consensus sequence. No polymorphic clone was represented more than once in the dual infection. In the individual with K65R alone, that mutation was detected at 10 days postseroconversion at 0.4% frequency. A separate clone from that individual 10 days postseroconversion showed a low-frequency variant (1.6%) with 4 didanosine-resistant mutations. In the third individual, virus carrying the K70R mutation at frequencies of 5% to 40% was present only prior to seroconversion. In this sample as in the others, *env* diversity was not proportional to that of RT. The authors pointed out that during transmission the diversity of the RT, including variants with drug resistance mutations, may be more complex than predicted from the *env* sequencing studies to date.

Baseline Resistance

The impact of minor PI mutations on the risk for failure during initial ART was examined by Scherrer and colleagues in an analysis of the SHCS (Swiss HIV Cohort Study) between 1999 and 2010 (Abstract 717). Of 926 patients who started ART with a PI/r-based regimen and 254 who started with an unboosted PI-based regimen, researchers compared outcomes among patients with 0 and 1 or more minor PI mutations. Times to virologic suppression and to virologic failure were similar in both groups. There was no single minor PI mutation that was statistically significantly associated with treatment outcome. This analysis was offered as evidence that the presence of minor PI mutations should not influence PI use.

Baseline ART resistance among treatment-naive patients in Vietnam was analyzed by Nhung and colleagues

(Abstract 718). Among 140 treatment-naive adults beginning ART with an NNRTI-based regimen enrolled between 2008 and 2010 and followed longitudinally, the baseline prevalence of resistance to nRTI, NNRTI, and PI drugs was 3.6%, 5.7%, and 0%, respectively. At 12 months, 27% of patients experienced treatment failure. In the multivariable analysis, resistance mutations were independently associated with immunologic failure (OR, 6.8; 95% CI, 1.2-39), but no correlation with clinical or virologic failure outcomes was seen.

Among HIV-1 subtype C patients in South Africa in whom an initial tenofovir-containing regimen failed, high rates of the K65R mutations at the time of failure were reported by Sunpath and colleagues (Abstract 719). Among 585 patients initiated on an initial tenofovir-containing regimen between 2010 and 2011, virologic failure occurred in 33 patients (5.6%), of whom 18 (54.5%) were found to have the K65R mutation. The authors pointed out that this proportion exceeds reported rates of K65R mutation emergence in similar analyses of subtype B infection, and suggested that genotypic resistance testing be expanded to better understand this phenomenon and shape ART selection in developing countries where non-B subtype HIV is common.

Low-Level Viremia and Virologic Failure

Given the increasing availability of extremely sensitive viral load monitoring assays, clinicians and investigators have sought an understanding of the significance of variations in low-level viremia persisting in patients on ART. Cologni and colleagues explored this issue (Abstract 348). There were 1214 patients with HIV RNA levels less than 50 copies/mL who were prospectively enrolled and serially monitored every 4 months by high-resolution PCR with a lower level of quantification of less than 3 copies/mL. At baseline, HIV RNA level was less than 3 copies/mL in 71.5% of patients and between 3 copies/mL and 50 copies/mL in 28.5% of cases. Over the following 12-month period, 43 patients (3.6%) reached confirmed HIV-1 plasma RNA over 50 copies/mL (virologic failure). The risk of ART failure during the 4-month monitoring period was statistically significantly greater for patients with an HIV RNA level less than 3 copies/mL at 0.4% compared with 3.2% for patients with any value of low-level viremia ($P < .0001$; OR, 7.52; 95% CI, 3.8-15.0). Genotypic analyses revealed that in 13 patients (representing 30.2% of virologic failures), mutations emerged that were able to alter the efficacy of the current ART. NNRTI-based ART was associated with HIV RNA levels less than 3 copies/mL throughout the study period compared with such levels in those receiving a PI/r or a non-boosted PI, with 45.2%, 33.1%, and 29.8% of the groups having HIV RNA levels below 3 copies/mL, respectively ($P < .0001$).

In contrast, Charpentier and colleagues reported an absence of increased risk for virologic failure associated with low-level viremia (Abstract 349). The authors compared rates of virologic failure in 618 patients who maintained HIV RNA levels less than 20 copies/mL and 38 patients with at least 2 HIV RNA levels between 20 copies/mL and 50 copies/mL. The proportion of patients experiencing virologic failure was not statistically significantly different between the 2 groups (4% vs 8%, respectively; $P = .32$). There was also no difference in blips (isolated HIV RNA levels > 50 copies/mL) between the 2 groups (0.09 vs 0.17, respectively; $P = .07$).

Persistent low-level viremia between 50 copies/mL and 500 copies/mL has been associated with virologic failure. However, conventional genotypic analysis may not be routinely performed until HIV RNA levels are as high as 1000 copies/mL. Delaugerre and colleagues reported on the selection of drug resistance mutations in patients with HIV-1 RNA levels less than 500 copies/mL on at least 3 occasions during a period of 6 months or longer on the same ART (Abstract 347). Rates of occurrence and emergence of drug resistance mutations during periods of HIV RNA levels between 40 copies/mL and 500 copies/mL were calculated in 37 HIV-1-infected patients on ART. Overall, 11 of 37 patients (30%) acquired at least 1 (and up to 9) drug resistance mutation during the period of low-level viremia. New drug resistance mutations were detected for an nRTI in 6 patients, for an NNRTI in 1 patient, for a PI in 6 patients, and for raltegravir in 2 patients. During the period of low-level viremia, the median number of drugs associated with confirmed resistance increased from 4.5 to 6. The authors concluded that periods of HIV RNA levels less than 500 copies/mL are associated with increased risk for accumulation of new drug resistance mutations and advised consideration of genotypic analysis at lower HIV RNA levels.

Quantifying Combination ART Effects

Sampah and colleagues reported methods to quantify the antiviral effects of ART on wild-type and drug-resistant HIV-1 infection (Abstract 624). Using a single-round infectivity assay, inhibition of wild-type and drug-resistant HIV-1 infection in primary CD4+ T cells was assessed and compared based on single drugs and drug combinations. The intrinsic antiviral activity of ART drugs and drug combinations at clinical concentrations was calculated relative to an identified target level of viral inhibition associated with treatment success. Previously unappreciated complex nonlinear pharmacodynamics were observed for most antiretroviral drugs. For example, combinations of InSTIs with drugs from all other classes showed a multiplicative effect on viral inhibition, and ART agents binding to the same site showed an additive effect, with the notable exception of PIs. The analysis provided a framework for understanding synergistic and antagonistic interactions between ART combinations, revealed novel regimens with high activity, and allowed for predictions of which regimens retain

residual antiretroviral activity against resistant virus strains.

Nucleic Acid and Drug Resistance Testing (NAT+DR)

In an effort to develop lower cost HIV RNA and drug resistance testing in RLS, Tilghman and colleagues tested a combined screening method based on qualitative nucleic acid testing (NAT) followed by sequencing of RT to detect drug resistance (NAT + DR) (Abstract 683). Plasma was collected from participants receiving at least 6 months of ART in the San Diego primary infection cohort. HIV RNA extraction and reverse transcription were performed on pools of 5 samples each, followed by PCR amplification of a conserved region of HIV-1 RT and sequencing of this region to detect drug resistance-associated mutations. Of the 325 patient samples analyzed, 50 (15%) had HIV RNA levels of at least 50 copies/mL (median 181 copies/mL, range 50 copies/mL to 10,500 copies/mL), and 4 (1%) had virologic failure (defined as HIV RNA level ≥ 1,000 copies/mL). Of the 65 mini-pools tested, 3 yielded product after 1 PCR round, and 19 yielded product after both rounds. The NAT + DR assay was 100% sensitive in the detection of individual samples with HIV RNA levels of at least 1000 copies/mL, using 1 or 2 PCR rounds. Sequences were successfully generated from PCR product of all pools testing positive for the presence of HIV, 36% of which harbored at least 1 important drug resistance mutation. Based on assay costs, the NAT+DR method would have saved $34,310 over standard HIV RNA level testing and genotyping of samples with virologic failure.

CCR5 Coreceptor Antagonists and Resistance

Optimizing detection of entry inhibitor resistance is an important area of study that was covered in CROI session 123. McLaughlin and colleagues shared data on a novel codon-specific PCR-based (CS-PCR) assay to detect HIV-1 subtype B variants using CXC chemokine receptor 4 (CXCR4) (Abstract 712). Relatively inexpensive, the CS-PCR detects codons in HIV *env* V3 that contribute to the CXCR4 phenotype. Optimization was achieved using 77 first-round *env* samples with accompanying population sequences and previously generated pyrosequencing data. In 22 of 77 samples, CXCR4 populations were present at 2% or more of the viral population, and at less than 2% in 23 of 77 samples. No CXCR4 was detected in the remaining 32 samples. CS-PCR detected a primary CXCR4 codon in 20 of 22 samples with 2% or more CXCR4 for a sensitivity of 91.7%, and 4 of 23 low-level (2% or less) CXCR4 populations. The authors offered CS-PCR as a sensitive and economical future alternative to more costly and technically demanding pyrosequencing.

The use of UDS applied to entry inhibitors continues to be an important area of investigation. Hedskog and colleagues evaluated the longitudinal determination of coreceptor usage by using ultra-deep pyrosequencing to examine the V3 loop of the viral envelope to determine the presence of CXCR4 virus as minority variants during primary HIV infection (Abstract 572). Three patients who experienced a coreceptor switch from CCR5 to CXCR4 were analyzed longitudinally from primary infection until after the switch. Each sample produced 480 to 20,893 reads. In 1 individual, low-abundant CXCR4-using viruses were found during primary infection. However, phylogenetic analysis suggested that these viruses were genetically similar to the CXCR4 population detected after coreceptor switch. The investigators offered this as evidence that CXCR4 populations develop from the CCR5 population of each patient during the course of infection.

NNRTI Resistance

Several abstracts explored issues of resistance to NNRTIs. Rimsky and colleagues reported on a week-96 resistance analysis of pooled rilpivirine and efavirenz phase III trials in treatment-naive HIV-infected adults (Abstract 708). Rilpivirine-based and efavirenz-based regimens each resulted in 78% response rate at 96 weeks. The week-96 analysis revealed virologic failures in 14% (96 of 686) of rilpivirine and 8% (52 of 682) of efavirenz regimens. Beyond week 48, increases in virologic failures were similar in both rilpivirine and efavirenz groups. Mutations associated with nRTI resistance emerged more frequently in rilpivirine than in efavirenz virologic failures in the week-96 analysis (56% vs 26%). The most frequently emerging NNRTI and nRTI resistance-associated mutations were E138K and M184I, respectively, in rilpivirine virologic failures, and K103N and M184V, respectively, in efavirenz virologic failures. E138K and M184I was the most frequent combination of resistance-associated mutations among rilpivirine virologic failures (22%). Among patients with baseline HIV RNA level of 100,000 copies/mL or below (low baseline viral load), there were 8% (28 of 368) rilpivirine virologic failures versus 6% efavirenz virologic failures, although a higher proportion of virologic failure in the rilpivirine (21%) than in the efavirenz group (9%) was observed in patients with baseline viral load greater than 100,000 copies/mL (high baseline viral load). In both treatment groups, the proportion of virologic failures with emergent resistance-associated mutations was lower among patients with low rather than high baseline viral load. Of 81 rilpivirine virologic failures, 35 showed resistance to rilpivirine by phenotyping at virologic failure. Of these 35 virologic failures, 16 (46%) were cross-resistant to nevirapine, 30 (86%) to efavirenz, and 32 (91%) to etravirine. Phenotypic resistance and NNRTI cross-resistance were less frequent in patients with low baseline HIV RNA levels than those with high baseline HIV RNA levels.

Hu and colleagues presented data on the effect of RT mutations E138K and M184I/V on rilpivirine susceptibility and viral fitness of HIV-1 (Abstract 706). Mutations at RT codons 138 and 184 were introduced by site-directed mutagenesis of cloned wild-type NL4-3 RT. Infectious recombinant viruses were generated. Viral infectivity and fitness profile, as well as susceptibility

to rilpivirine and replication capacity, were determined. The E138K and E138K/M184V mutations conferred a 2.2-fold increase in 50% inhibitory concentration (IC_{50}) for rilpivirine compared with wild-type mutations, and the E138K/M184I mutation conferred a 4.9-fold increase. The relative infectivity and fitness profiles of the mutants compared with wild-type virus over a range of drug concentrations showed that the E138K/M184I mutant had a replicative advantage over the E138K/M184V mutant at higher rilpivirine concentrations tested (0.0625 nM to 1 nM). The E138K/M184I mutant had similar advantage over the E138K/M184V mutant over the range of drug concentrations tested (0.0039 nM to 0.25 nM for rilpivirine; 0.39 µM to 25 µM for lamivudine). The authors concluded that the higher level of resistance and greater relative replication of the E138K/M184I mutant than of the E138K/M184V mutant likely explained the frequent association of E138K with M184I in HIV-1 strains derived from patients with virologic failure in clinical trials of rilpivirine.

Anta and colleagues assessed the presence of the rilpivirine resistance mutations E138K and M184I in HIV-1-infected patients in whom NNRTI regimens failed in the ResRIS (Spanish AIDS Research Network national drug-resistance database) (Abstract 710). Investigators analyzed 8200 RT genotypes from 5873 different HIV-infected patients, of which 1064 belonged to patients in whom NNRTI therapy had failed. Codon 138 mutants were found to be very rare. However, almost 20% of patients on failing NNRTI regimens would be considered rilpivirine resistant, as a result of other mutations (V90I, V108I, E138K, V179I, and Y181C) that are more often selected when failing nevirapine or etravirine rather than efavirenz, therefore limiting the sequential use of these drugs.

Similarly, Sungkanuparph and colleagues reported on a study to assess primary HIV-1 drug resistance-associated mutations to efaviranez, etravirine, nevirapine, and rilpivirine among ART-naive HIV-1-infected patients in Thailand from 2007 to 2010 (Abstract 709). HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. The 466 patients included in the analysis had HIV-1 subtypes CRF01_AE (86.9%), B (8.6%), and other recombinants (4.5%). The prevalence of patients with efavirenz, etravirine, nevirapine, and rilpivirine resistance were 3.2%, 1.3%, 3.2%, and 1.5%, respectively. All patients who had etravirine resistance and all patients with rilpivirine resistance (except for 1 patient with E138G) also had efavirenz and nevirapine resistance. The most common NNRTI resistance-associated mutations observed, in descending order of frequency, were V179D, V106I, Y181C, K103N, and V108I. The authors commented that after a decade of ART scale-up in Thailand, there is substantial primary resistance to NNRTIs, which extends to second- as well as first-generation NNRTIs. The authors urged interventions to prevent the transmission of HIV drug resistance and continue surveillance for primary HIV drug resistance.

Integrase Resistance

Several presentations were dedicated to resistance in the InSTI class. Lin and colleagues explored differences in kinetics and magnesium dependency of binding of first-, second- and third-generation HIV InSTIs to wild-type versus raltegravir-resistant G140S/Q148H HIV (Abstract 690). Equilibrium binding and kinetic studies were consistent with a previously proposed 2-step binding model, with equilibrium largely controlled by magnesium-dependent steps and final equilibrium controlled by conformational changes around the terminal deoxyribose/adenine. The relative participation of each binding step to total binding was found to be altered with binding of third-generation InSTIs to the raltegravir-resistant mutant, offering insight into inhibitors that remain potent against raltegravir resistant HIV.

Understanding barriers to resistance among second-generation InSTIs was the focus of several studies. Winters and colleagues reported on the development of InSTI resistance mutations in patients on elvitegravir-containing failing regimens (Abstract 701). RNA was isolated at 52 timepoints from 10 HIV-infected patients with suboptimal virologic response enrolled in the phase II study exploring the use of elvitegravir/r in the absence of PIs in heavily treatment-experienced patients. Plasma samples were taken at baseline and sequentially during treatment (from week 2 until week 48). Genotypic analysis was performed. Primary InSTI drug resistance mutations in clones were determined using the Stanford HIV Drug Resistance Database. Although all patients had PI, nRTI and NNRTI resistance–associated mutations at baseline, no baseline InSTI mutations were detected. During elvitegravir treatment, patients developed primary InSTI resistance–associated mutations as early as 2 weeks after initiation of treatment. Two to 6 strains of different primary InSTI resistance–associated mutations appeared during early treatment failure, predominantly as single mutations. The prevalence of these strains fluctuated over time. New strains, or strains with new combinations of InSTI resistance–associated mutations, developed over time. Final virologic failure timepoints (weeks 14 to 48) typically showed a dominant strain that did not possess InSTI resistance–associated mutations found in the early time points and had multiple mutations or a single N155H mutation. The authors suggested that in patients with multiclass drug resistance, elvitegravir treatment can select for a number of distinct InSTI-resistant strains and this can be a highly dynamic process. Early identification of treatment failure may restrict the emergence of strains highly resistant to elvitegravir.

Oliveira and colleagues explored novel mutational changes involved in delayed emergence of resistance to the investigational drug dolutegravir in HIV-1 B and non-B subtypes during in vitro selection (Abstract 692). Findings included supportive evidence that dolutegravir possesses a high genetic barrier to resistance and that HIV subtype may play a role in the mutant selection process.

Raltegravir was the first InSTI

approved by the FDA to treat HIV-1. Although recent research suggests efficacy against HIV-2 as well, HIV-2 susceptibility data are limited. Smith and colleagues reported on the phenotypic susceptibility to raltegravir and genetic pathways to InSTI resistance in HIV-2 (Abstract 700). Using site-directed mutagenesis of an HIV-2 molecular clone, 11 HIV-2 integrase mutants were constructed. Replication capacity and raltegravir susceptibility of the resultant variants were assessed. Raltegravir had comparable activity against wild-type HIV-1 and HIV-2. Varying degrees of resistance were appreciated with various known mutations. Amino acid changes Q148K, Q148R, N155H, and T97A + N155H individually conferred moderate resistance to raltegravir, whereas the combination of replacements G140S + Q148R and Q148R + N155H imparted high-level raltregravir resistance (> 100-fold). In contrast, mutations T97A, G140S, Y143C, Q148H, and T97A + Y143C had no substantial effect on raltegravir sensitivity in HIV-2 (≤ 3-fold increase in median effective concentration [EC_{50}]).

With regard to replication capacity, mutations Y143C, T97A + Y143C, Q148H/K/R, and Q148R + N155H showed statistically significant declines in infectious titers, and G140S partially restored the replication defect imposed by the Q148R substitution. Authors concluded that clinical studies of raltegravir for treating HIV-2 infection are indicated. Kobayashi and colleagues studied the in vitro antiviral activity of dolutegravir against raltegravir-resistant HIV-2 mutants (Abstract 691). Dolutegravir showed limited cross-resistance to raltegravir-resistant HIV-2 and substantial loss of activity was not appreciated in the presence of single or even combinations of mutations. The authors suggested that dolutegravir might be useful for the treatment of raltegravir- and elvitegravir-resistant HIV-2 infection.

PIs and Resistance

In an effort to further understand HIV-2 susceptibility to PIs, Raugi and colleagues examined the effects of single amino acid changes in protease on the susceptibility of HIV-2 to 3 PIs: darunavir, saquinavir, and lopinavir (Abstract 697). Using site-directed mutagenesis, 8 protease mutants (V10I, I32V, V47A, I54M, I82F, I84V, L90M, and L99F) were constructed and single-cycle assays were used to quantify the sensitivity of wild-type and mutant HIV-2 strains to each PI in culture. Relative to the wild-type strain, the I54M variant of HIV-2 protease showed moderate resistance to darunavir, whereas the L90M mutant was resistant to saquinavir, and the V47A mutant was resistant to lopinavir. Interestingly, 3 amino acid substitutions tested increased the sensitivity of HIV-2, including the V47A (saquinavir), I32V (darunavir), and I82F (both saquinavir and darunavir).

This year's CROI offered many insights into recent advances in antiretroviral therapy. Promising phase I studies of a tenofovir prodrug which may have increased virologic efficacy with decreased systemic toxicity and infusions of zinc-finger nuclease-modified, CCR5-disrupted autologous T cells, were paired with phase III trials of a new once-daily combination of elvitegravir/cobicistat/tenofovir/emtricitabine and dolutegravir, an investigational InSTI. Data regarding mortality on long-term ART emphasized the near-normal lifespan available to individuals initiating ART at CD4+ counts over 500 cells/μL, while highlighting the persistent challenge of early diagnosis and treatment so that people can realize these benefits. Evidence continued to mount regarding the appropriate use and clinical relevance of low-frequency resistance mutations. Finally, data from RLS revealed advances in treatment scale-up and improvements in median CD4+ cell count at ART initiation, although gaps in linkage to care and adherence fatigue remain to be addressed.

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