Advances in Antiretroviral Therapy

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The 16th Conference on Retroviruses and Opportunistic Infections maintained its tradition of being recognized as the preeminent forum for detailing the state-of-the-art of antiretroviral therapy. Abundant new and updated information was presented on investigational drugs, approaches to the management of treatment-naïve and -experienced patients, the use of drugs for prevention of mother-to-child HIV-1 transmission, and antiretroviral drug resistance. Of particular note were the continued advances in antiretroviral treatment and research emanating from resource-limited settings and the presentation of the results of 2 much-anticipated, phase III trials of interleukin-2, ESPRIT (Evaluation of Subcutaneous Proteinkin in a Randomized International Trial) and SILCAAT (Subcutaneous, Recombinant Human Interleukin-2 in HIV-Infected Patients With Low CD4+ Counts Receiving Active Antiretroviral Therapy).

Investigational Drugs

Cytochrome P450 3A Inhibitors

GS-9350. Data were presented on 2 compounds that inhibit cytochrome P450 3A (CYP3A) without having antiretroviral activity. These are being developed as alternatives to ritonavir. Mathias and colleagues presented data on GS-9350 (Abstract 40). This compound provides potent irreversible inhibition of CYP3A4 without demonstrable anti-HIV-1 activity. GS-9350 exhibited more specificity for CYP3A inhibition than ritonavir (ie, other CYP450 enzymes were less likely to be inhibited by GS-9350). GS-9350 did not inhibit normal lipid accumulation or glucose uptake in adipocytes, suggesting less potential for metabolic abnormalities. Numerous single-dose and multiple-dose studies of GS-9350 were performed in HIV-uninfected participants. These studies evaluated change in clearance of midazolam, which is metabolized by CYP3A. GS-9350 100 mg once daily or 200 mg once daily provided similar reductions in midazolam clearance to that provided by ritonavir 100 mg daily. A coformulated 4-drug tablet of elvitegravir (an investigational integrase inhibitor)/tenofovir/emtricitabine/GS-9350 is currently under development. Elvitegravir concentrations with GS-9350 150 mg once daily resemble those with ritonavir 100 mg once daily. The exposure to emtricitabine was increased 20% in the presence of elvitegravir/GS-9350, and the tenofovir exposure was bioequivalent. There were no substantial safety concerns; a drug-related, transient grade 3 elevation of alanine aminotransferase developed in 1 participant. Phase II studies of the 4-drug tablet are under way.

SPI-452. Gulnik and colleagues from Sequoia Pharmaceuticals presented data on SPI-452, a potent inhibitor of CYP3A4 with no discernible inhibition of HIV-1 protease or HIV-1 replication (Abstract 41). SPI-452 was generally well tolerated, with no serious adverse events. No changes in serum lipid levels were observed compared with placebo. The half-life was 15 hours to 20 hours at higher doses. SPI-452 enhanced the pharmacokinetic profile of darunavir and atazanavir in a manner similar to that of ritonavir in historical cohorts. However, the investigators did not evaluate a concurrent ritonavir control group.

Entry Inhibitors


CD4-BFFI. Jekle and colleagues presented data on a novel entry inhibitor, CD4-BFFI (Abstract 551). This compound combined an anti-CD4 monoclonal antibody (anti-CD4 mAb 6314) with a fusion inhibitor (T-651). The antiviral activity of the bifunctional compound was much greater than that of the individual components.

Reverse Transcriptase Inhibitors

CMX157. CMX157 is a lipod conjugate of tenofovir that leads to 30-fold higher intracellular levels of tenofovir-diphosphate. Lanier and colleagues tested the hypothesis that CMX157 also enters peripheral blood mononuclear cells by associating with HIV-1 virions (Abstract 556). They found that approximately 30,000 molecules of CMX157 were associated with each virion compared with approximately 100 molecules of tenofovir. HIV-1 virions inoculated with CMX157 were less infectious, whereas inoculation with tenofovir did not affect infectivity.

Ribonuclease H inhibitors. Current reverse transcriptase inhibitors (ie, nucleoside analogue reverse transcriptase inhibitors [nRTIs] and nonnucleoside analogue reverse transcriptase inhibitors [NNRTIs]) work by inhibiting the polymerase function of reverse transcriptase. Williams and colleagues presented data on inhibitors of ribonuclease (RNase) H, a portion of reverse transcriptase responsible for cleaving the RNA template following synthesis of the first DNA strand during reverse transcription (Abstract 559). They...
found a series of inhibitors of RNase H that also showed antiviral effects in the cell culture; however, these compounds had a modest therapeutic index with relatively low levels of 50% cellular cytotoxicity.

Integrase Inhibitors

QNL111. Two abstracts presented preclinical data on integrase inhibitors with novel mechanisms of action. Raltegravir and the investigational integrase inhibitor elvitegravir work by inhibiting the strand-transfer reaction. Thibaut and colleagues presented data on a new quinoline family of compounds (Abstract 553). These compounds are integrase binding inhibitors that inhibit the 3' processing step. The lead compound, QNL111, has a 50% inhibitory concentration for HIV replication of 800 nM and retained activity against isolates resistant to raltegravir and elvitegravir.

Integrase-LEDGF/p75 inhibitors. Lens epithelium-derived growth factor p75 (LEDGF/p75) acts as a tethering factor for integrase to chromatin. Benarous and colleagues screened 70,000 compounds for their ability to inhibit the LEDGF/p75-integrase interaction (Abstract 555). Compounds were identified that inhibited HIV-1 integration without inhibiting the catalytic activity required for the strand-transfer reaction, and these compounds retained activity against HIV-1 resistant to raltegravir.

Maturation Inhibitors

MPC-9055. Baichwal and colleagues presented data on a new maturation inhibitor, MPC-9055 (Abstract 561). This compound blocks the processing of the CA-SP1 Gag intermediate to the mature protein CA. Serial passage experiments identified a single amino acid substitution in Gag, A364V, which induced resistance. Beelen and colleagues presented the safety, tolerability, and pharmacokinetics of MPC-9055 in HIV-uninfected volunteers (Abstract 570). Fifty-five participants received a single dose of MPC-9055, and 20 received placebo. There were more treatment-emergent events in those receiving MPC-9055. All events were mild except for 1 instance of moderate diarrhea. The half-life was 23 hours to 42 hours, and absorption was increased in the presence of meals. Further multiple-dose studies are planned.

Dolabella diterpene. Abreu and colleagues presented data on dolabella diterpene (DT), a natural extract from the Brazilian brown algae Dictyota pflaffii (Abstract 562). This compound had a median effective concentration versus HIV-1 of 30 nM. The researchers found unprocessed p55 Gag and partially processed p41 and p25 precursors accumulating in HIV virions in the presence of DT. This compound retained activity against viral strains with high-level resistance to bevirimat, another HIV maturation inhibitor. The authors concluded that DT binds to a different site than bevirimat, possibly the p55 Gag precursor.

RNA Interference
tat and vif short-interfering RNA. Choi and colleagues evaluated RNA interference in a humanized mouse model of HIV (Abstract 564). They noted that HIV is highly mutable and it is likely that escape mutants would develop if only a single RNA sequence were targeted. They targeted 2 highly conserved regions in tat and vif genes by administering synthetic short-interfering RNA specific to those sequences that was delivered using a T-cell-targeting, single-chain antibody as a carrier. This was highly effective at suppressing viral replication, with all mice having plasma HIV-1 RNA levels below the limit of detection throughout the treatment period.

Immune-Based Therapies

Interleukin-2

Results were presented of 2 large, long-awaited, randomized, controlled, open-label trials evaluating the efficacy of subcutaneous administration of interleukin-2 (IL-2) in combination with antiretroviral therapy versus antiretroviral therapy alone (Abstracts 90aLB, 90bLB). Both studies used occurrence of an opportunistic disease or death as the primary endpoint.

ESPRIT. The ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) enrolled 4111 participants from 25 countries. Eligible enrollees had a CD4+ count above 300 cells/µL. Participants either continued or initiated combination antiretroviral therapy at study entry. Patients who underwent randomization to receive IL-2 were given 3 5-day cycles of 7.5 mIU subcutaneously, twice daily at 8-week intervals. Additional cycles were recommended as needed to keep the CD4+ cell count greater than twice the baseline, or at least 1000 cells/µL.

At study entry, the median CD4+ count was 457 cells/µL; the median nadir CD4+ count was 197 cells/µL; 80% had a plasma HIV-1 RNA level at or below 500 copies/mL; 26% had a prior AIDS-defining illness; 19% were female; 24% were nonwhite; and mean age was 41 years. The median length of follow-up was 6.9 years.

Patients in the IL-2 group maintained, on average, a CD4+ count 160 cells/µL higher than that of the control group. However, the rate of primary endpoints (opportunistic disease or death) did not differ between the IL-2 and control groups (1.13 vs 1.21 events/100 person-years, respectively; hazard ratio [HR], 0.93; 95% confidence interval [CI], 0.75–1.16). Similarly, the rate of death and serious non-AIDS events was similar between the 2 groups. There were more grade 4 (ie, life-threatening) events in the IL-2 group (HR, 1.23; 95% CI, 1.00–1.51). The difference in grade 4 events was driven by administration and injection site reactions, psychiatric events, and vascular events such as deep venous thrombosis.

SILCAAT. The SILCAAT (Subcutaneous, Recombinant Human Interleukin-2 in HIV-Infected Patients With Low CD4+ Counts Receiving Active Antiretroviral Therapy) study randomized 1695 participants from 25 countries. Eligible participants had a CD4+ count between 50 cells/µL and 299 cells/µL. Patients either continued or initiated combination antiretroviral therapy at study entry. Patients who underwent randomization to receive IL-2 were given 3 5-day cycles of 7.5 mIU subcutaneously, twice daily at 8-week intervals. Additional cycles were recommended as needed to keep the CD4+ cell count greater than twice the baseline, or at least 1000 cells/µL.
antiretroviral therapy. Participants who underwent randomization to IL-2 were given 6 5-day cycles of 4.5 mIU subcutaneously, twice daily at 8-week intervals. Additional cycles were recommended as needed to keep the CD4+ count 150 cells/µL greater than baseline.

At study entry, the median CD4+ count was 202 cells/µL; the median nadir CD4+ count was 60 cells/µL. 81% had a plasma HIV-1 RNA level at or below 500 copies/mL; 32% had a prior AIDS-defining illness; 17% were female; 20% were nonwhite; and mean age was 40 years. The median length of follow-up was 7.6 years.

Participants in the IL-2 group maintained, on average, a CD4+ count 57 cells/µL higher than that of the control group. However, the rate of primary endpoints (opportunistic disease or death) did not differ between the IL-2 and control groups (HR, 0.91; 95% CI, 0.70−1.18). The rate of grade 4 adverse events was similar between the IL-2 and control groups (HR, 1.11; P = .30). However, the rate of grade 4 events in the first year after randomization was statistically significantly higher in the IL-2 group (HR, 2.02; P = .01). Taken together, data from these studies show that the CD4+ count gain associated with IL-2 administration does not translate into reduced clinical events. In addition, there appears to be an increased risk of serious adverse events attributed to IL-2 therapy.

**Interleukin-2 and inflammatory markers.** Porter and colleagues evaluated the effect of subcutaneous IL-2 treatment on levels of high-sensitivity C-reactive protein (hsCRP) and D-dimer (Abstract 533). They found that the levels of these molecules were increased statistically significantly in patients with (n = 54) and without (n = 19) virologic suppression. The median level of hsCRP increased from 1.15 mg/L to 63.8 mg/L and 1.01 mg/L to 62.6 mg/L, respectively, in these groups. The D-dimer level increased from below 0.20 µg/mL to 0.79 µg/mL and from 0.27 µg/mL to 2.91 µg/mL, respectively (P < .001 for all 4 comparisons). The increases were transient and returned to baseline within 1 month after IL-2 administration.

**Genetically modified CD4+ cells.** There has been a recent appreciation that HIV-1 infection causes a profound depletion of gut lymphoid tissue early in the infection. Collman and colleagues presented data on 12 patients receiving suppressive antiretroviral therapy in an ongoing phase II/III open-label trial of genetically modified autologous CD4+ cells (Abstract 83). The CD4+ cells are collected and transduced ex vivo with lentiviral vector encoding a 937-nucleotide antisense construct to the env gene. Six infusions of 10^5 cells each were given to each participant, followed by interruption of antiretroviral therapy in 7 patients. The genetically modified CD4+ cells persisted in the plasma after treatment interruption. The researchers also found that these cells migrated to the rectal epithelium and persisted after treatment interruption. Future analyses will evaluate whether this therapy can partially control HIV-1 replication.

**Histone deacetylase inhibitors.** Histone deacetylase (HDAC) is a key enzyme for maintaining latency of HIV-1 infection in resting CD4+ cells. HDAC inhibitors are being pursued in an effort to clear this latent reservoir of HIV-1 infection as a key step toward HIV-1 eradication. Hong and colleagues presented data on 2 synthetic HDAC inhibitors, CGMC0005 and CGMC0006 (Abstract 418). They found that both compounds induced HIV-1 replication, as measured by p24 antigen production in latently infected cell lines with 50% effective doses of 0.11 µM and 0.04 µM, respectively.

**Clinical Trials of Antiretroviral Therapy in Treatment-Naive Patients**

**Antiretroviral Therapy in Patients with Tuberculosis**

Swaminathan and colleagues presented data on 127 HIV-1-infected patients with active tuberculosis (Abstract 35). Participants received standard short-course tuberculosis therapy, and underwent randomization after the 2-month induction phase to receive either open-label efavirenz (600 mg once daily) or nevirapine (400 mg once daily after 2 weeks of 200 mg once daily); both were given with didanosine and lamivudine. The median age was 35.5 years, and 99 (78%) were men. The median CD4+ count was 84 cells/µL, and the median plasma HIV-1 RNA level was 310,000 copies/mL. After 24 weeks of antiretroviral therapy, more patients in the efavirenz group achieved a plasma HIV-1 RNA level below 400 copies/mL (50/59 patients, 85%) compared with 38 of 57 patients (67%) in the nevirapine group (P = .038). Moreover, there were 5 deaths in the nevirapine group and none in the efavirenz group. These results along with the virologic data prompted the Data and Safety Monitoring Board to stop the study early.

**Efavirenz Versus Lopinavir/Ritonavir; Didanosine Plus Zidovudine Versus Stavudine Plus Lamivudine**

Ratsela and colleagues presented data from Phidisa II, a randomized, factorial-design study of lopinavir/ritonavir (lopinavir/r) versus efavirenz and didanosine plus zidovudine versus stavudine plus lamivudine (Abstract 594). Eligible participants had a CD4+ count below 200 cells/µL or a prior clinical AIDS diagnosis. The primary endpoint was AIDS or death. The study included 1771 patients with a median CD4+ count of 106 cells/µL and plasma HIV-1 RNA level of 144,000 copies/mL. There was no statistically significant difference in the primary endpoint between the groups. There did not appear to be any statistically significant difference in the rates of virologic suppression between the efavirenz and lopinavir/r groups 48 weeks after randomization (66% vs 65%, respectively). Consistent with prior studies, the CD4+ count responses were greater in the lopinavir/r group than in the efavirenz group: an increase of 317 cells/µL at 3 years versus 272 cells/µL, respectively (P = .004). Although the rates of the primary endpoint did not differ between the didanosine plus zidovudine recipients and the stavudine plus lamivudine recipients, the CD4+ cell count and virologic data favored stavudine plus lamivudine.
Clinical Trials of Antiretroviral Therapy in Treatment-Experienced Patients

PRO 140

PRO 140 is a humanized monoclonal antibody that blocks binding of HIV to CCR5. Thompson and colleagues presented data from a phase Ila study that evaluated the subcutaneous administration of PRO 140 in HIV-1-infected subjects (Abstract 571a). Eligible participants had plasma HIV-1 RNA levels above 5000 copies/mL, CCR5-using HIV, and no antiretroviral therapy within the past 12 weeks. The 44 patients underwent randomization to receive placebo, PRO 140 162 mg once weekly, PRO 140 324 mg once every 2 weeks, or PRO 140 324 mg once weekly. The mean change in plasma HIV-1 RNA level was +0.15, −0.75, −1.2, and −1.51 log₁₀ copies/mL in the 4 groups, respectively. Administration site reactions were mild or absent in all subjects. The authors concluded that this study supported further investigation of infrequent subcutaneous administration of PRO 140.

Antiretroviral Treatment Strategies

Intensification

New antiretroviral assays can measure plasma HIV-1 RNA level with a lower limit of detection of 1 copy/mL; many patients taking combination antiretroviral therapy with virologic suppression using standard ultrasensitive assays have detectable residual viremia using this 1 copy/mL assay. The source of the residual viremia is not known. If it represents complete cycles of virus replication, then suppressing this residual viremia could hasten the clearance of the latent reservoir. Several studies examined whether this residual viremia could be suppressed by intensifying combination antiretroviral regimens with a new drug.

Raltegravir. Jones and colleagues enrolled 5 participants with a mean level of residual viremia of 1.9 copies/mL (Abstract 423b). Participants added raltegravir to their existing antiretroviral drug regimen for 30 days. The mean level of viremia at the end of the intensification period was 5.2 copies/mL, which was not statistically significantly different from the level prior to intensification.

Buzon and colleagues randomly assigned 65 participants with plasma HIV-1 RNA levels below 50 copies/mL to either intensify their existing regimen by adding raltegravir for 48 weeks (n = 44) or continue their regimen without intensification (n = 21) (Abstract 423a). Intensification did not alter the level of proviral DNA. There was a statistically significant increase in episomal HIV-1 DNA at week 2, but the difference was not seen after week 2. The authors concluded that this increase provided evidence for continued de novo viral infection with suppressive antiretroviral therapy (ie, raltegravir prevented integration of viral DNA from ongoing replication that was instead converted to episomal DNA).

Enfuvirtide. Gandhi and colleagues evaluated the latent reservoir directly in participants initiating an intensive first antiretroviral regimen of enfuvirtide, saquinavir/ritonavir, and tenofovir/emtricitabine (Abstract 424). They enrolled 19 patients with a median CD4+ count of 262 cells/µL and plasma HIV-1 RNA level of 4.8 log₁₀ copies/mL. Seventeen participants achieved a plasma HIV-1 RNA level below 50 copies/mL within 48 weeks, 9 of whom remained on enfuvirtide for at least 48 weeks and were included in the primary analysis. The primary endpoint was the proportion of latently infected, resting memory CD4+ cells, which was measured every 24 weeks. This endpoint did not change over the 96 weeks of study follow-up. The primary endpoint was similar to that observed in previous studies using less intensive regimens. The authors concluded that other strategies were needed to decrease the size of the latent reservoir.

Switching from Lopinavir/Ritonavir to Raltegravir

Eron and colleagues presented data on 2 large, randomized, placebo-controlled trials of switching lopinavir/ritonavir to raltegravir in patients with successful virologic suppression on a lopinavir/ritonavir-containing regimen (SWITCHMRK-1 and SWITCHMRK-2) (Abstract 70aLB). Eligible participants had a plasma HIV-1 RNA level below 50 copies/mL (or < 75 copies/mL by branch DNA assay) for at least 3 months and had not received lipid-lowering therapy for at least 12 weeks. Patients with prior virologic failure were not excluded, and enrollees were not required to be intolerant of lopinavir/ritonavir. The primary objectives of these studies were to show a decrease in lipid levels 12 weeks after randomization and noninferiority of continued virologic suppression 24 weeks after randomization for those switching to raltegravir compared with those continuing treatment with lopinavir/ritonavir.

In SWITCHMRK-1 and -2, 348 and 354 patients, respectively, underwent randomization. The mean age was 44 years and 42 years; 79% and 78% were men; and 28% and 53% were nonwhite, respectively. The mean CD4+ count at baseline was approximately 480 cells/µL in both studies. The median number of prior antiretroviral drugs taken was 5 and 6, respectively.

Levels of triglycerides, total fasting cholesterol, and non-high-density-lipoprotein (HDL) cholesterol decreased statistically significantly with the switch to raltegravir (P < .001 for each comparison in both studies). However, switching to raltegravir did not achieve noninferiority for maintaining virologic suppression compared with continuing lopinavir/ritonavir treatment. The proportion of patients with a plasma HIV-1 RNA level below 50 copies/mL at week 24 was 81% for the raltegravir group versus 87% for the lopinavir/ritonavir group (treatment difference, −6.6%; 95% CI, −14.4 to +1.2) in SWITCHMRK-1 and 88% versus 94% (treatment difference, −5.8%; 95% CI, −12.2 to +0.2) in SWITCHMRK-2. When combining both studies, the proportion with plasma HIV-1 RNA level below 50 copies/mL at week 24 was statistically significantly lower for the raltegravir group. Of the 32 patients with virologic failure, 27 (84%) reported that this was not their first antiretroviral regimen, and 18 (66%) reported having experienced virologic failure with a prior regimen.
Antiretroviral Treatment in Resource-Limited Settings

The conference kicked off this year with an impressive keynote address, the N’Galy-Mann Lecture by Gray and McIntyre, who detailed their experiences in HIV clinical research and care in Soweto, South Africa (Abstract 18).

Treatment Scale-Up in Resource-Limited Settings

Gray, McIntyre, and colleagues founded the Perinatal HIV Research Unit (PHRU) in Soweto in 1996 and have expanded the program over the past 2 decades to include 50 active research programs in HIV prevention, care, treatment, and the sociobehavioral and economic impacts of the HIV epidemic. Relevant to this article on antiretroviral therapy advances, the PHRU launched its first antiretroviral treatment trial in 1996, as a means of providing access to antiretroviral therapy in a setting where it was otherwise unavailable. The PHRU began offering antiretroviral therapy outside of clinical trials in 2001 with funds from the French government, and the program expanded with PEPFAR (President’s Emergency Plan for AIDS Relief) support to include 45,000 people started on antiretroviral therapy in a collaboration of 10 partners and 45 sites over 4 provinces.

Gray and McIntyre also have been leaders in clinical research, including an ongoing treatment trial comparing nurse-monitored with physician-monitored treatment; the CHER (Children with HIV Early Antiretroviral Therapy) study, which showed that early treatment of infants with antiretroviral therapy led to substantial improvements in mortality; and the OCTANE (Optimal Combination Therapy After Nevirapine Exposure) study (Abstract 94LB), which demonstrated improved outcomes with lopinavir/boosted antiretroviral therapy compared with nevirapine-based antiretroviral therapy in women exposed to single-dose nevirapine for prevention of mother-to-child transmission (PMTCT) of HIV.

Throughout this time, the South African President, Thabo Mbeki, and his Health Minister, Manto Tshabalala-Msimang, waged both active and underground campaigns to question the role of HIV as the cause of AIDS and the efficacy of antiretroviral treatment. Their interventions ranged from restructuring the South African Medicines Control Council when it refused to approve treatment trials of a toxic industrial solvent as antiretroviral therapy to casting personal aspersions against the PHRU and its researchers. Despite this opposition, the support of outside funding institutions and a strong treatment advocacy program led by activists allowed the PHRU to continue functioning and the South African national antiretroviral treatment program to grow.

Gray and McIntyre concluded by discussing current challenges in confronting the South African epidemic, including the 1.8 million people estimated to be in need of antiretroviral therapy by 2011, looming funding shortfalls, and the need for increased outreach and care for older women and men who have sex with men (MSM). They also emphasized the continuing need for activist scientists, in the mold of N’Galy and Mann, who recognize the importance of human rights in the response to the HIV epidemic.

Coutinho spoke on the “Limits and Realities of Antiretroviral Therapy Scale-up” (Abstract 12). He noted that, currently, 30% of individuals needing antiretroviral therapy receive it, and antiretroviral therapy scale-up does not keep pace with the rate of new infections. The success in global expansion of access to antiretroviral therapy, as indicated by the increase from the 200,000 people living with HIV or AIDS receiving treatment in resource-limited settings (RLS) in 2002 to the over 3 million receiving treatment in 2007, is largely the result of increased access by the “low-hanging fruit,” Coutinho’s term for individuals who were waiting for initial antiretroviral therapy in situations where capacity was in place to deliver care. The challenge of the future is to scale up antiretroviral therapy access in locations without current capacity and for people living with HIV who are less likely to seek care.

As signs of progress in antiretroviral therapy scale-up, Coutinho cited the increases in life expectancy in Botswana, reductions in mortality among HIV-infected people after antiretroviral therapy became available in Uganda, and the fact that task-shifting, which places more responsibilities for patient care onto nurses and midwives, does not appear to have affected treatment outcomes. However, he drew attention to the global underperformance of PMTCT, with only 18% of women giving birth in low- and middle-income countries being tested for HIV, and in diagnosis of HIV and tuberculosis coinfection, with only 12% of people with tuberculosis being tested for HIV. Rates of mortality (20%) and loss to follow-up (15% – 17%) in the first 5 years of treatment are also unacceptably high. Coutinho concluded by anticipating future challenges to the second, more difficult stage of antiretroviral therapy scale-up, including the dramatic lack of human resources and laboratory capacity, the geographic distribution of people in need of care, the transition to more complicated cases and second-line therapy, and the gap between current funding and that needed for universal access to antiretroviral therapy.

Coetzee and Boulle presented data on the response to the HIV epidemic in Cape Town, South Africa, including information relevant to antiretroviral treatment scale-up (Abstract 58). Currently, antiretroviral therapy coverage in Cape Town is estimated at 60% using antiretroviral therapy scale-up, including the dramatic lack of human resources and laboratory capacity, the geographic distribution of people in need of care, the transition to more complicated cases and second-line therapy, and the gap between current funding and that needed for universal access to antiretroviral therapy.

Coetzee also noted a decrease in HIV prevalence among 15- to 19-year-olds over the past 5 years and a decrease
in the incidence of sexually transmitted infections in Khayelitsha, from 0.27 per 100,000 people in 2003 to 0.13 per 100,000 people in 2007.

Schechter described the Brazilian response to scale-up as a reflection of its political climate (Abstract 59). The military dictatorship that led the country from 1964 to 1985 gave way to a democratic regime and a national universal health care system. When the first AIDS cases were recognized in the 1980s and private insurance companies refused to provide care, activists joined ranks to fight for HIV care. In 1988, nongovernmental organizations and government bodies began to work together to provide universal HIV care, and the government began to provide zidovudine therapy. Currently, the Brazilian program offers numerous options for initial therapy, including both protease inhibitor (PI)- and NNRTI-based regimens, most of which are provided by the government regardless of patient insurance status.

The responsibilities for laboratory testing and other services in Brazil are divided between state and federal government, which leads to disparities between states in access to services. Antiretroviral therapy outcomes are comparable to those in more resource-rich settings: baseline CD4+ count at antiretroviral therapy initiation is 200 cells/µL, and 72% of individuals starting antiretroviral therapy achieve plasma HIV-1 RNA levels of less than 50 copies/mL after 6 months. Non-HIV-related causes of death are on the rise; the cardiovascular disease death rate is growing at 8% per year for those with HIV and 0.8% per year for those without. The majority of people in Brazil are unaware of their HIV serostatus, and the complexity of injection drug use and MSM subepidemics that vary by region requires targeted outreach campaigns.

Schechter concluded by summarizing future improvements necessary for the continued success of the Brazilian program, including an expanded definition of universal access from antiretroviral therapy alone to treatment as 1 component in a sustainable access-to-treatment effort, an increase in targeted and operational research efforts, and improvement in the speed and quality of actions by institutional review boards.

Mohammed and colleagues presented data on the unmet need for HIV testing, care, and treatment in Kenya derived from the 2007 Kenya AIDS Indicator Survey (KAIS) (Abstract 137LB). This survey included individuals 15 years to 64 years of age in a stratified, 2-stage, cluster-sample design covering 8 provinces and urban and rural areas. Household- and individual-level data were collected between August 2007 and December 2007 in the form of interviews and venous blood samples. Overall response rates were 97% for households, 91% for individuals, and 80% for blood samples. HIV prevalence results showed that 8.4% of women and 5.4% of men were HIV seropositive, with peak prevalences in age 30 years to 34 years for women and 40 years to 44 years for men. These results were similar to the country’s 2003 estimates of HIV prevalence. Uncircumcised men were 3.4 times more likely to have HIV infection than were circumcised men. Of HIV-seropositive adults in the sample, 84% were unaware they were infected, and 44% of married or cohabiting HIV-infected persons had an uninfected partner. An estimated 120,000 HIV-infected adults with CD4+ counts below 250 cells/µL are in need of antiretroviral therapy, but among those who knew their HIV serostatus and were eligible for antiretroviral therapy, 92% were receiving therapy. The authors concluded that low awareness of HIV serostatus was the major barrier to universal antiretroviral therapy access in Kenya, and that this type of nationally representative survey is essential for monitoring the epidemic and improving the national response.

Primary Treatment Outcomes in Resource-Limited Settings

Lockman and colleagues presented the results of the OCTANE A5208 Trial, a randomized trial examining whether prior single-dose nevirapine treatment for PMTCT limits future virologic response to nevirapine-containing antiretroviral regimens (Abstract 94LB). Two concurrent trials were conducted within the study design. The first compared lopinavir/ritonavir plus tenofovir/emtricitabine with nevirapine plus tenofovir/emtricitabine in 240 women with prior receipt of single-dose nevirapine and was designed to show superiority of the lopinavir/ritonavir regimen. The second trial compared the same 2 regimens in 500 women without single-dose nevirapine exposure and was designed to show equivalence; it is still under way.

Inclusion criteria for the first trial were as follows: CD4+ count less than 200 cells/µL, no prior antiretroviral therapy except single-dose nevirapine at least 6 months previously, up to 10 weeks of prior zidovudine treatment, and an estimated creatinine clearance rate of over 60 mL/min. Ten sites in 7 countries in sub-Saharan Africa enrolled study participants, and the primary endpoint was death or time to virologic failure. The authors defined virologic failure as a confirmed plasma HIV-1 RNA level less than 1 log10 copies/mL below the baseline level after 12 weeks of treatment or more than 400 copies/mL after 24 weeks of antiretroviral therapy and conducted a primary intent-to-treat analysis.

In October 2008, the Data and Safety Monitoring Board recommended cessation of the first trial after enrollment of 243 women into the trial, when the median duration of follow-up was 73 weeks. There were no statistically significant differences in the baseline characteristics of participants by treatment group. Overall, 41 women reached the primary endpoint described above: 31 in the nevirapine group and 10 in the lopinavir/ritonavir group. The estimated HR of reaching the endpoint in the nevirapine group was 3.55 (95% CI, 1.71–7.34 compared with the lopinavir/ritonavir group), and the difference in risk between the 2 groups was established by 24 weeks on treatment. The rate of treatment discontinuation was statistically significantly higher in the nevirapine group: 38 women (31%) compared with 6 (5%) in the lopinavir/ritonavir group (HR, 7.43; 95% CI, 3.14–17.59), and common reasons for discontinuation were adverse events and virologic failure.

Drug resistance testing showed that 14% of women had nevirapine resis-
tance mutations at baseline, and the median time since last single-dose nevirapine exposure was 11 months in women with nevirapine resistance compared with 17 months in women without resistance ($P = .024$). Although the proportion of women with virologic failure or death was higher among those with nevirapine resistance, there was a trend toward virologic failure or death in the nevirapine group, even among women without nevirapine resistance ($P = .057$). The data, when broken down by time since last single-dose nevirapine exposure, also suggested that the difference between treatment groups was lower among women starting antiretroviral therapy more than 24 months after receiving single-dose nevirapine. The authors noted that these findings may not be applicable to women who received PMTCT regimens other than single-dose nevirapine, and that treatment success in the lopinavir/ritonavir trial was higher than anticipated.

Lawn and colleagues presented data, now published, on the changes in mortality risk associated with CD4+ count response to antiretroviral therapy in South Africa (Abstract 140). They sought to determine mortality estimates for those receiving antiretroviral therapy for longer than 1 year and examined the relationships between risk of mortality, pretreatment CD4+ counts, and CD4+ count response to antiretroviral therapy, using an observational cohort in the Gugulethu township of Cape Town, South Africa. National antiretroviral therapy guidelines allow for initiation of a first-line NNRTI-based regimen when the CD4+ count falls below 200 cells/µL or a patient has WHO clinical stage IV HIV disease.

Between 2002 and 2007, 2,434 patients in the cohort initiated antiretroviral therapy; 67% were women, median age was 33 years, median CD4+ count at antiretroviral therapy initiation was 101 cells/µL, and 23% of patients had WHO stage IV disease. The median follow-up period was 1.3 years, or 3,155 person-years, and 192 deaths occurred during this time. Cumulative mortality was 8.4% at 12 months and 13.2% at 48 months. Mortality at 48 months for patients with a pretreatment CD4+ count less than 100 cells/µL was 16.7%, almost twice that of those with CD4+ counts above 100 cells/µL (9.5%). Comparing these data with data from the ATHENA (AIDS Therapy Evaluation in the Netherlands) cohort, mortality for similarly stratified patients is approximately 3 times higher across all strata for the Gugulethu cohort.

To determine the effect of CD4+ count response to antiretroviral therapy on mortality, the investigators calculated the amount of person-time accrued in each 100 cells/µL stratum of CD4+ counts during the follow-up period. The mortality rate in deaths per 100 person-years for CD4+ counts below 200 cells/µL to 1.2 to 2.7 deaths per 100 person-years for CD4+ counts above 200 cells/µL. In the multivariate analysis, age, WHO stage IV disease, and plasma HIV-1 RNA level above 400 copies/mL all correlated with mortality, but the largest correlation was for CD4+ count response strata. For those whose CD4+ count remained between 0 cells/µL and 49 cells/µL, the risk ratio for mortality was 11.63 (95% CI, 3.95−34.29) compared with those whose CD4+ counts rose to over 500 cells/µL during antiretroviral therapy.

The authors also note that most of the person-time accrued in the lower CD4+ count strata occurred in the first 12 months after antiretroviral therapy initiation, and that those with a pretreatment CD4+ count below 100 cells/µL made up 53% of the time accrued with CD4+ counts less than 200 cells/µL compared with those with a pretreatment CD4+ count above 100 cells/µL (28% of the time with CD4+ count < 200 cells/µL). Thus, the patients with the lowest baseline CD4+ counts spent more time with levels below 200 cells/µL and had higher risks of death. The investigators concluded that these data argue for an increase in the CD4+ cell count threshold at which to initiate antiretroviral therapy.

Brinkhof and colleagues from the ART-LINC (Antiretroviral Therapy in Lower Income Countries Collaboration) compared the mortality of HIV-infected patients initiating antiretroviral therapy with that of the general population in sub-Saharan Africa (Abstract 141). They pooled data from 15,249 HIV-infected, treatment-naive patients age 16 years or older receiving treatment in 5 public antiretroviral therapy clinics in South Africa (2 sites), Zimbabwe, Cote d'Ivoire, and Malawi. The baseline characteristics of this cohort included the following: 67% women, median age of 34 years, median CD4+ count of 107 cells/µL (interquartile range [IQR], 46−175), and 85% with WHO stage III or IV disease. In the first 2 years of antiretroviral therapy, 1,177 deaths occurred. The overall cumulative mortality at 2 years was 11.7% (95% CI, 11.1−12.3). The authors used data on expected mortality from the WHO Global Burden of Disease estimates and used multiple imputation to account for missing baseline CD4+ counts, clinical stage, and mortality outcome in those lost to follow-up.

The investigators found that the excess mortality rate in those who initiated antiretroviral therapy with CD4+ counts below 25 cells/µL was 17.5 per 100 person-years (95% CI, 14.5−21.1) compared with the general population in sub-Saharan Africa. Among patients who initiated antiretroviral therapy with CD4+ counts above 200 cells/µL and WHO clinical stage III/IV, excess mortality was only 1 per 100 person-years (95% CI, 0.55−1.81) and decreased to 0.29 per 100 person-years (95% CI, 0.17−0.49) if they survived the first year of antiretroviral therapy. When accounting for prognostic factors such as age, sex, baseline CD4+ count, and clinical stage of HIV disease, half of the patients in the cohort had excess rates of 5 to 20 deaths per 100 person-years, and half had fewer than 5 additional deaths per 100 person-years. The investigators concluded that, though excess mortality rates are high in these cohorts, those without severe disease who survive into the second year with therapy have mortality rates comparable to those of the general population.

Mwango and colleagues described preliminary outcomes of Zambia's decision to introduce tenofovir as a component of an initial antiretroviral regimen in their national antiretroviral therapy pro-
mortality is highest in the first 3 months for the tenofovir group, and 2.7 per 100 person-years: 29.3 (95% CI, 27.0–31.6) for the stavudine group, and 11.7 (95% CI, 10.9–12.5) for the tenofovir group. Switches were highest in the zidovudine group in the first 90 days of therapy. Mortality after 90 days of treatment was 5.2 per 100 person-years for the stavudine group, 4.8 per 100 person-years for the tenofovir group, and 2.7 per 100 person-years for the zidovudine group. After adjustment for baseline demographic and disease stages, the HR values for mortality in each treatment group had overlapping 95% CI values. The only statistically significant subgroup difference was between patients who started with zidovudine and then switched and those who remained with initial tenofovir. The adjusted HR for 90-day mortality in the zidovudine-switch group was 2.41 (95% CI, 1.41–4.12). The proportion of patients with creatinine clearance rates below 50 mL/min at 6 months and 12 months increased in all 3 groups, but no statistically significant difference between treatment groups was found. Overall, the investigators noted that high levels of drug switching and relatively short follow-up periods in this observational study made the outcomes difficult to interpret, but they concluded that tenofovir-based regimens were more easily tolerated and did not lead to higher rates of renal insufficiency.

**Treatment Outcomes in Children in Resource-Limited Settings**

Moultrie and colleagues presented clinical, virologic, and immunologic outcome data from HIV-infected children receiving antiretroviral therapy at the Harriet Shezi Children’s Clinic, a tertiary academic center that is the largest public pediatric HIV site in South Africa (Abstract 97). The study observed a prospective cohort of 2102 children under 15 years of age who initiated antiretroviral therapy between April 2004 and March 2008. Only children with at least 1 post-antiretroviral therapy initiation follow-up visit were included, but the authors noted that 18% of children who had accessed care at the clinic at least once were lost to follow-up before antiretroviral therapy initiation.

At the time of antiretroviral therapy initiation, median age was 4.3 years, 51% were boys, median CD4+ cell percentage was 11.5% IQR, 6.9–16.2), and 29% of patients were receiving treatment for tuberculosis. The median number of months of follow-up during antiretroviral therapy for members of the cohort was 17 (IQR, 5.7–29.2). Most of the 152 deaths in the cohort occurred in the first 3 months of antiretroviral therapy and in children under 18 months of age. Factors predicting mortality in a multivariate analysis included severe underweight for age, high plasma HIV-1 RNA level, concurrent tuberculosis treatment, and younger age. By 18 months of antiretroviral therapy, 90% of the children had plasma HIV-1 RNA levels below 400 copies/mL, and statistically significant improvements in immunologic status were seen in the first 12 months of antiretroviral therapy. The investigators concluded that the timing of antiretroviral therapy initiation in the clinic is not optimal, that mortality is highest in the first 3 months after initiation of antiretroviral therapy but excellent virologic and immunologic outcomes are seen, and that improved outcomes would be likely if infants received earlier diagnoses and initiated antiretroviral therapy earlier.

Pendergast and colleagues examined the impact of co-infection with cytomegalovirus (CMV) on HIV disease (Abstract 93LB). Compared with resource-rich settings, where 36% to 65% of children are infected with CMV by adolescence, 85% of infants in RLS are infected. The investigators collected data as a part of the PEHSS (Pediatric Early Highly Active Antiretroviral Therapy Sexually Transmitted Infections Study) in Durban, South Africa, which is examining the efficacies of 3 antiretroviral therapy management strategies in infants to determine the impact of CMV co-infection on HIV disease progression in these infants. They used CMV real-time polymerase chain reaction assays to test plasma samples from infants 3 months to 4 months of age enrolled in the PEHSS and compared the pre–antiretroviral therapy rate of CD4+ percentage decline from birth in CMV-seropositive and CMV-seronegative infants. Samples were available for 54 of the 63 infants in the cohort, and 59% were CMV seropositive by 3 months to 4 months of age.

CMV-seropositive infants were more likely to be breastfed (53%) than CMV-seronegative infants (18%; \( P = .01 \)), but otherwise there were no statistically significant differences in baseline characteristics between the 2 groups. There was also no difference overall in morbidity and mortality and no difference in weight or head circumference at 12 months. There was a trend toward more failure to thrive in the CMV-seropositive infants (43% compared with 17% in CMV-seronegative infants; \( P = .07 \)). For CD4+ percentage, the decline was twice as rapid in CMV-seropositive as in CMV-seronegative infants: 10.5% per month and 5.0% per month, respectively ( \( P = .007 \)). This led to progressive differences in CD4+ percentage in each group, which persisted up to 12 months post–antiretroviral therapy initiation ( \( P = .004 \)). No difference was seen in the absolute nadir CD4+ count, which the authors speculate may be related to observed CD8+ cell count increases seen...
in CMV-seropositive infants. This raises the question of whether the CD4+ percentage decline in CMV-seropositive infants is a result of primary CMV infection rather than HIV infection, and the authors are pursuing this question in further studies.

Outcomes of Second-Line Antiretroviral Therapy in Resource-Limited Settings

The number of studies presenting data on treatment strategies and second-line antiretroviral treatment outcomes in RLS increased substantially this year. Keiser and colleagues presented data on transitions to second-line antiretroviral therapy and mortality from the ART-LINC Collaboration of the International Epidemiological Databases to Evaluate AIDS (leDEA) (Abstract 608). The investigators examined data from 20,113 patients from 17 HIV and AIDS treatment programs in Africa, South America, and Asia who met the following criteria: 16 years of age or older, antiretroviral therapy–naive, NNRTI-based initial antiretroviral therapy, and at least 6 months of follow-up data available. Second-line regimens were defined as a change after 6 months of initial therapy with an NNRTI-based regimen to a PI-based regimen along with a change of at least 1 nRTI. WHO guidelines for immunologic or virologic failure of antiretroviral therapy were used.

The primary outcome was change to a second-line regimen, which occurred in 576 patients after a median of 20 months, with a rate of 2.4 per 100 person-years, determined by Kaplan-Meier analysis. A low CD4+ count at baseline was the most important predictor of switching to second-line therapy. The availability of plasma HIV-1 RNA level monitoring at the treatment site was a predictor of an earlier switch to second-line therapy, and CD4+ counts at treatment change were higher at sites with plasma HIV-1 RNA monitoring (161 cells/μL) than at those without it (102 cells/μL; P < .001). Mortality was higher in patients who continued their initial antiretroviral regimens after meeting WHO criteria for immunologic or virologic failure (10.7 deaths/100 person-years; 95% CI, 7.3–15.6) than in patients who switched (5.1 deaths/100 person-years; 95% CI, 3.2–8.1) and in those whose initial regimen did not fail (2.9 deaths/100 person-years; 95% CI, 2.4–5.7). The authors concluded that the rate of change to second-line therapy was relatively low and that patients continuing treatment with failing initial regimens are at risk for increased mortality.

Hosseinipour and colleagues determined outcomes of standard second-line antiretroviral therapy (zidovudine, lamivudine, tenofovir, and lopinavir/r) in a cohort of 109 patients receiving treatment in 2 clinics in Malawi (Abstract 605). Participants qualified for second-line therapy if they met clinical or immunologic criteria for antiretroviral therapy failure and had failure confirmed with a plasma HIV-1 RNA level above 400 copies/mL. Of 109 patients whose antiretroviral therapy failed by these criteria, 101 initiated second-line therapy, 5 (5%) died before initiating second-line therapy, and 10 (9%) died within 6 months of initiating second-line therapy. Factors associated with death by 6 months in a multivariate analysis included clinical failure (odds ratio [OR], 3.47; 95% CI, 1.14–10.59) and body mass index below 18.5 kg/m² (OR, 4.43; 95% CI, 1.15–17.12). Among the 101 patients initiating second-line antiretroviral therapy, approximately 70% had achieved plasma HIV-1 RNA levels below 400 copies/mL by 12 months despite baseline nRTI-associated resistance mutations, and 19% experienced grade 3 or grade 4 antiretroviral therapy toxicities.

Fox and colleagues presented findings on the outcome of second-line antiretroviral therapy from a prospective cohort of 382 patients in South Africa (Abstract 606). They included all patients older than 18 years from a single treatment site in Johannesburg who initiated antiretroviral therapy with a standard initial treatment regimen followed by the public-sector second-line antiretroviral therapy regimen of zidovudine, didanosine, and lopinavir/r. The mean increase of CD4+ count 12 months after initiation of second-line therapy was 118 cells/μL, and 89% were alive and in care. Seventy-eight percent of the cohort achieved a plasma HIV-1 RNA level below 400 copies/mL. Predictors of plasma HIV-1 RNA level below 400 copies/mL at 1 year after adjusting for age, sex, adherence to initial treatment, and total duration of antiretroviral therapy include the following: at least 1 single antiretroviral drug substitution before initiation of second-line antiretroviral therapy (adjusted HR, 0.71; 95% CI, 0.56–0.89); and a duration of more than 3 weeks between a repeat plasma HIV-1 RNA level above 1000 copies/mL and the change to second-line antiretroviral therapy (adjusted HR, 0.60; 95% CI, 0.43–0.83).

Ive and colleagues presented data on time from virologic failure to antiretroviral therapy regimen change from the same clinic population in Johannesburg, South Africa (Abstract 607). Of 8649 patients receiving initial antiretroviral therapy with 2 nRTIs and 1 NNRTI, 428 had confirmed virologic failure, with 2 consecutive plasma HIV-1 RNA level measurements above 1000 copies/mL. Of these, 190 (44%) patients initiated second-line antiretroviral therapy within 3 months of meeting criteria for virologic failure, 141 (33%) patients initiated second-line antiretroviral therapy more than 3 months after failure, and 95 (22%) never switched antiretroviral therapy regimens. Survival analysis was used to estimate the median time to switching from first- to second-line therapy after confirmed virologic failure: 200 days (95% CI, 174–280). The authors are currently exploring reasons for the delay in transition from first- to second-line treatment after virologic failure.

Evaluation of World Health Organization Clinical and Immunologic Criteria for Treatment Failure of Initial Antiretroviral Therapy

In 2006, the WHO published guidelines for antiretroviral therapy in adults and adolescents that included clinical, CD4+ count, and virologic definitions of treatment failure for patients receiving an initial antiretroviral regimen. Three groups evaluated the value of these criteria in clinical cohorts of HIV-seropositive individuals in RLS.

Reynolds and colleagues examined the WHO immunologic criteria for anti-
retroviral therapy failure among adults receiving treatment from the Rakai Health Sciences Program in Uganda (Abstract 144). Participants were included who initiated antiretroviral therapy between June 2004 and September 2007 and who had at least 6 months of follow-up care. Patients in the program had both virologic and immunologic monitoring every 6 months, and they switched to second-line therapy if their plasma HIV-1 RNA level surpassed 10,000 copies/mL after an adherence intervention. Of 1133 participants with a median 20 months of follow-up who were receiving an initial NNRTI-based regimen, 125 (11%) met immunologic criteria for antiretroviral therapy failure. 80 (7%) met virologic failure criteria of plasma HIV-1 RNA levels above 10,000 copies/mL, but only 18 (1.6%) met both criteria.

The sensitivity and specificity of the immunologic criteria for determining virologic failure were 23% and 90%, respectively. These did not change when the criteria for virologic failure were made more stringent: having 2 samples with plasma HIV-1 RNA levels above 400 copies/mL. Using the WHO criteria, only 107 of the 125 immunologic failures were considered virologic failures, and the authors determined that use of the immunologic criteria was both insensitive for identifying failures and would lead to cost increase because of unnecessary switches in those whose regimens were not failing.

Two other groups examined the same question in settings where plasma HIV-1 RNA level monitoring is not routinely available and is used only to confirm treatment failure in patients meeting WHO clinical or immunologic criteria for failure. Rewari and colleagues collected data from the Indian national antiretroviral therapy program; they found that referral of cases of suspected treatment failure to a panel of clinical experts was useful in helping determine the need for antiretroviral therapy regimen changes but that 24% of patients meeting clinical or immunologic criteria for antiretroviral therapy failure had plasma HIV-1 RNA levels below 400 copies/mL (Abstract 609).

Etiob and colleagues conducted a retrospective analysis of data from the ACTION (AIDS Care and Treatment in Nigeria) project, and found that the immunologic criteria had a sensitivity and specificity of 81% and 49%, respectively, for predicting virologic treatment failure of an initial regimen (Abstract 610). Predictors of virologic failure in the cohort included previous antiretroviral therapy exposure, having more than 1 “first-line” regimen, CD4+ count below 200 cells/µL before plasma HIV-1 RNA level testing, and a decrease in weight by more than 10%.

**Laboratory Monitoring in Resource-Limited Settings**

Several poster sessions were dedicated to laboratory monitoring in RLS. Session 185, “Methods of Viral Load Monitoring,” covered improved detection of diverse viral subtypes (Abstracts 998, 1004, 1005), comparisons of new and old plasma HIV-1 RNA level detection methods (Abstracts 999–1002), and the efficiency of pooled viral load testing (Abstract 1003). Session 186 included 4 presentations (Abstracts 1006–1009) evaluating the use of dried blood spots for plasma HIV-1 RNA level testing in RLS.

**Breastfeeding and Mother-to-Child Transmission**

**Review of Transmission of HIV Through Breastfeeding**

HIV transmission from mother to child continues to result in as many as 700,000 infections yearly worldwide. In a plenary presentation, Stringer elaborated on the contribution of breastfeeding to transmission of HIV to children, outlining the field’s current understanding as well as emerging strategies to prevent MTCT through breast milk (Abstract 127).

Breastfeeding now contributes up to one-third of HIV transmissions to children, and the risk of transmission to infants is approximately 0.5% per month while breastfeeding, according to the BHITS (Breastfeeding and HIV International Transmission Study). Studies aimed at supporting formula feeding in lieu of breastfeeding (Mashi Study) or early weaning (Zambia Exclusive Breastfeeding Study, ZEBS) have proven disappointing, revealing that reductions in HIV transmission are offset by infectious complications and mortality.

Specific strategies under investigation include providing antiretroviral therapy for lactating women and antiretroviral prophylaxis for the breastfeeding infant. Promising data from Kenya (Kisumu Breastfeeding Study) and Tanzania (MITRA-Plus Study) have shown that antiretroviral therapy for mothers beginning in the third trimester and continuing through breastfeeding (6 months) greatly reduces the transmission of HIV attributable to breastfeeding compared with historical controls.

Postexposure prophylaxis of infants is a second promising option for prevention of mother-to-child transmission. The SWEN (Six Week Extended Nevirapine) and PEPI (Post-Exposure Prophylaxis for Infants) studies, presented at the 2008 (15th) conference last year, also offer a hopeful alternative to avoidance of breastfeeding by treating babies with antiretroviral therapy during the breastfeeding period.

Stringer emphasized that most of the transmissions occur among women with more advanced HIV disease, suggesting that maternal treatment is advisable for women who require treatment, whereas infant prophylaxis is a suitable alternative for women in whom antiretroviral therapy is not yet indicated for their own health. The ongoing study comparing maternal treatment to infant prophylaxis during breastfeeding, the BAN (Breastfeeding, Antiretrovirals, and Nutrition) study, should inform us as to the best practice when completed in summer 2009.

**Impact of Breastfeeding on Infant Health in Resource-Limited Settings**

ZEBS. Follow-up analysis on the ZEBS was presented by Kuhn and colleagues (Abstract 963). Previously published results of this randomized study of 958 HIV-infected postpartum women in Zambia concluded that there was no HIV-free survival benefit of abrupt weaning at 4 months of age compared with gradual weaning at 6 months. The
authors evaluated the contribution of ineffective abrupt weaning on the lack of difference in the 2 groups. HIV-free survival was the same among women who effectively weaned (83.9%) as it was for those who continued to breastfeed (84%); however, an interaction was detected between the results of weaning and the severity of maternal disease. Among babies of asymptomatic women with CD4+ counts above 350 cells/µL, those who weaned were at increased risk of HIV infection or death (HR, 3.01; 95% CI, 1.16–7.81) compared with those who did not. Among babies of symptomatic women or those with CD4+ counts below 350 cells/µL, in the absence of antiretroviral therapy, early weaning improved rates of HIV-free survival. The authors concluded that for asymptomatic women with higher CD4+ counts, breastfeeding has better HIV-free survival than early abrupt weaning.

Also from the ZEBS trial, Arpadi and colleagues evaluated the impact of the timing of breastfeeding cessation on infant growth; they found that continued breastfeeding mitigated growth faltering among uninfected children born to HIV-infected mothers (Abstract 962). They analyzed the weight and height for age z-scores in 593 uninfected infants who were still alive and breastfeeding at 4 months of age and found statistically significantly more rapid declines in weight for age z-scores in children who were weaned.

Breastfeeding and malaria. Breastfeeding was associated with protection against malaria in a Ugandan prospective study of children born to HIV-infected women, as presented by Hornsby and colleagues (Abstract 961). The authors observed 200 HIV-exposed children who were tested for HIV at 6 weeks of age, and again at 6 weeks after breastfeeding cessation. Exclusive breastfeeding for 6 months was recommended for HIV-seronegative infants, and breastfeeding for as long as possible was recommended in HIV-infected infants. Breastfeeding cessation occurred by 15 months of age in 185 children (median age of cessation, 7.2 months). The analysis was stratified by HIV serostatus and age (ages 6 to < 9 months, and 9–15 months). Children breastfed from ages 9 months to 15 months had a statistically significant reduction in malaria as diagnosed by fever and blood smear (HR, 0.51; P = .003). Results remained unchanged after stratification by HIV serostatus.

Maternal Health During Breastfeeding

Mashi study. To further explore previous reports of the negative impact of breastfeeding on maternal health, Lockman and colleagues presented results of a randomized trial from the Mashi study in Botswana on the effect of breastfeeding versus formula feeding on maternal HIV progression, mortality, and micronutrient levels (Abstract 176). From 2001 to 2003, 1200 women were randomly assigned to either breastfeeding with zidovudine for infant prophylaxis and weaning at 6 months or formula feeding; participants were followed up for a median of 54 months. Antiretroviral therapy was available for women who met WHO criteria. Baseline characteristics were similar between the 2 groups. The primary endpoint of time to CD4+ count below 200 cells/µL, AIDS, or death was reached in 34% of breastfeeding women and 28% of women randomly assigned to use of formula, but the difference was not statistically significant (P = .08).

Further exploration of this trend revealed that the largest contributor to this composite endpoint was CD4+ count below 200 cells/µL, which occurred in 25% of women randomly assigned to breastfeeding and only 21% in the formula-feeding group. Rates of death were the same in the 2 groups at 3%. Statistically significant predictors of the composite endpoint in multivariate analysis were CD4+ count at or below 350 cells/µL (P < .01) and plasma HIV-1 RNA level at or above the median (P < .01), and any education was protective (P = .04). No statistically significant differences in micronutrient levels were appreciated between the 2 groups. The authors did detect statistically significantly higher high-sensitivity C-reactive protein levels in the breastfeeding group (P < .01) and plan to explore reasons for this in future studies. The authors concluded that breastfeeding was not associated with an increase in maternal mortality.

Weight status during breastfeeding. ZEBS investigators Murnane and colleagues examined the role of breastfeeding on maternal weight loss (Abstract 982). The authors reported on a secondary analysis of weight and weight changes in 758 HIV-infected, breastfeeding women. HIV-infected, breastfeeding women were more likely to gain rather than lose weight from 4 months to 24 months postpartum and, in particular, women with low baseline body mass index values or lower CD4+ counts were spared substantial weight loss.

Prevention of Mother-to-Child Transmission

Third-trimester testing for HIV-seronegative women. Routine testing for HIV during early pregnancy has become the standard of care, allowing for early diagnosis in pregnancy and affording the greatest likelihood for prevention of transmission of HIV from mother to child. Increasingly, it has been recognized that repeat testing in the third trimester is advisable in high-risk populations. Lu and colleagues reviewed their data on HIV incidence among women in Botswana during pregnancy and during breastfeeding in the first year postpartum (Abstract 91). They found that 470 of 1090 (43%) infant infections were attributable to incident HIV in the mother during pregnancy or breastfeeding, emphasizing the importance of third-trimester and postpartum retesting.

Antiretroviral therapy to prevent transmission through breastfeeding. Taha and colleagues presented updated analyses of the PEPi-Malawi study on the effect of maternal antiretroviral therapy on postnatal HIV-1 transmission after cessation of extended infant antiretroviral prophylaxis (Abstract 92). The study involved breastfeeding, HIV-seronegative infants who underwent randomization at birth to receive single-dose nevirapine and 1 week of zidovudine (control regimen), control regimen plus extended daily nevirapine until 14 weeks, or
control regimen plus extended daily nevirapine and zidovudine for 14 weeks. Previous study results showed that extended nevirapine treatment resulted in a 67% reduction in transmission to infants during their time on treatment. This analysis examined the association of maternal antiretroviral therapy use on transmission to infants after cessation of the infant prophylaxis.

Of 2318 infants not infected at 14 weeks, 130 (5.6%) became infected at follow-up between 14 weeks and 24 months. Women with a CD4+ count below 250 cells/µL were eligible to receive antiretroviral therapy. After adjusting for infant prophylaxis, the HR for transmission to infants was lower in the antiretroviral therapy-eligible women who received antiretroviral therapy (adjusted HR, 0.18; 95% CI, 0.07 – 0.44) than in the antiretroviral therapy-eligible women who did not receive treatment.

The authors also noted that the transmission risk was lower in antiretroviral therapy–ineligible women (ie, CD4+ count at or above 250 cells/µL) who did not receive antiretroviral therapy (adjusted HR, 0.35; 95% CI, 0.25 – 0.50) than in antiretroviral therapy–eligible women who did not receive antiretroviral therapy. There was no statistically significant difference in HIV transmission comparing women who received antiretroviral therapy and those who were antiretroviral therapy–ineligible and did not receive treatment. The authors recommended treating antiretroviral therapy–eligible women with antiretroviral therapy to prevent MTCT through breastfeeding.

Preventing resistance after PMTCT. Given its prolonged half-life, nevirapine, when given as a single dose, is associated with subsequent NNRTI resistance in 24% to 76% of women. This limits subsequent antiretroviral therapy options. Numerous presentations reviewed strategies to reduce the rate of NNRTI resistance after single-dose nevirapine for PMTCT. Van Dyke and colleagues reviewed the IMPAACT (International Maternal Pediatric Adolescent AIDS Clinical Trials) P1032 phase II study of the incidence of resistance mutations in HIV-infected Thai women receiving a single intrapartum dose of nevirapine followed by either 7 days or 30 days of a PI-based regimen or 30 days of an nRTI-based regimen compared with a historical control group (Perinatal HIV Prevention Trial-2, PHPT-2) (Abstract 95aLB). They included data from 169 nonbreastfeeding women older than 18 years. Women were randomly assigned to receive 7 days of zidovudine, didanosine, and lopinavir/ritr (group A), 30 days of zidovudine and didanosine alone (group B), or 30 days of zidovudine, didanosine, and lopinavir/ritr (group C). At 2 weeks or 6 weeks of follow-up, all 3 groups had statistically significantly less nevirapine resistance (A, 3.6%; B, 7.1%; C, 5.3%) than historical control patients did (31%); however, the 3 groups were not statistically significantly different from each other. The authors concluded that 7 days of combination antiretroviral therapy prevents most nevirapine resistance from emerging after single-dose nevirapine administration and has little toxicity.

Lallemant and colleagues presented data on the use of an nRTI tail to prevent NNRTI resistance emergence after use of single-dose nevirapine (Abstract 95bLB). They enrolled 222 pregnant, antiretroviral therapy-naive women (“cases”) with a CD4+ count above 250 cells/µL who were treated with zidovudine in the third trimester, followed by intrapartum single-dose nevirapine, followed by 1 month of zidovudine and didanosine. The authors compared the rate of nevirapine resistance-associated mutations in these cases with that of matched control patients treated with single-dose nevirapine alone in the earlier PHPT-2 trial. Both groups underwent consensus sequencing and oligonucleotide ligation assay for K103N, Y181C, and G190A mutations. Cases and matched control patients had similar baseline characteristics. Combining results of both assays, resistance mutations were found in 1.8% of cases and 20.7% of control patients. Such data offer a practical solution in RLS, where alternatives to single-dose nevirapine and its associated resistance are not yet readily available.

Response to nevirapine-containing regimens after single-dose nevirapine.

Jourdain and colleagues reported on follow-up analysis of PHPT-2 and found a continued, statistically significant effect of single-dose nevirapine after 4 years of therapy (Abstract 954). Women from the initial cohort received zidovudine from 28-weeks’ gestation followed by either single-dose nevirapine or placebo. Women with a CD4+ count at or below 250 cells/µL who subsequently received a nevirapine-based regimen were included in the analysis of resistance. There were 221 single-dose nevirapine–exposed women and 48 unexposed women. At 4 years, 41% of exposed women and 23% of unexposed women (P = .02) experienced treatment failure (plasma HIV-1 RNA level > 400 copies/mL after 4.5 months, CD4+ count < 50 cells/µL at 6 months, switch to PI, or death). The authors noted that the risk of failure in exposed women decreased with time from delivery to treatment initiation (adjusted OR, 0.93/month increment; P = .001).

Lockman and colleagues also provided long-term maternal and pediatric virologic outcomes of nevirapine-based antiretroviral therapy following receipt of single-dose nevirapine or placebo in the Mashi study in Botswana (Abstract 955). Three hundred sixty women were randomly assigned to receive either single-dose nevirapine or placebo and were observed for a median of 42 months. Virologic failure was more common when initiating antiretroviral therapy less than 6 months after single-dose nevirapine than when initiating it at or more than 6 months later (P = .003). Among HIV-infected children, 56 started antiretroviral therapy. The highest rates of virologic failure were identified in infants who received single-dose nevirapine born to mothers who also received single-dose nevirapine.

Table 1 summarizes some of these presentations on antiretroviral treatment in RLS.

**Resistance**

Transmitted Drug Resistance

Review of transmitted drug resistance. Pillay lead a symposium on “HIV Drug Resistance and Treatment Response”
<table>
<thead>
<tr>
<th>Abstract No.</th>
<th>Study Title</th>
<th>Research Question</th>
<th>Study Design</th>
<th>Findings</th>
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<tr>
<td>Abstract 94LB.</td>
<td>Lopinavir/ritonavir + Tenofovir/emtricitabine is Superior to Nevirapine + Tenofovir/emtricitabine for Women with Prior Exposure to Single-Dose Nevirapine: A5208 (OCTANE)</td>
<td>Does single-dose nevirapine use for PMTCT limit future virologic response to nevirapine-containing regimens?</td>
<td>Multisite randomized control trial: Lopinavir/ritonavir + tenofovir/emtricitabine vs nevirapine + tenofovir/emtricitabine ( (n = 243) ) Botswana, Kenya, Malawi, South Africa, Uganda, Zambia, Zimbabwe</td>
<td>Trial stopped early by DSMB with median duration of follow-up at 73 weeks. Hazard ratio for reaching primary endpoint of death or virologic failure (confirmed plasma HIV-1 RNA level less than ( 1 \log_{10} ) copies/mL below baseline after 12 weeks of treatment or ( &gt; 400 ) copies/mL after 24 weeks of treatment) in nevirapine arm compared with lopinavir/ritonavir arm: 3.55 (95% CI, 1.71–7.34)</td>
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<tr>
<td>Abstract 140.</td>
<td>Changing Mortality Risk Associated with CD4 Cell Response to Long-Term ART: Sub-Saharan Africa</td>
<td>What is the relationship between mortality risk and CD4+ count response in those on antiretroviral therapy for more than 1 year?</td>
<td>Single-site observational cohort study ( (n = 2434) ) Cape Town, South Africa</td>
<td>Cumulative mortality: 8.4% at 12 months and 13.2% at 48 months 48-month mortality: baseline CD4+ &lt; 100 cells/µL, 16.7% baseline CD4+ &gt; 100 cells/µL, 9.5% Strongest predictor of mortality was on-treatment CD4+ count strata: CD4+ 0–199 cells/µL: mortality rate, 5.4–38.6 deaths/100 person-years; CD4+ 200–≥ 500 cells/µL: mortality rate, 1.2–2.7 deaths/100 person-years</td>
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<td>Abstract 143.</td>
<td>Randomized Trial of Trained Patient-Nominated Treatment Supporters Providing Partial Directly Observed ART in South African Adults Initiating HIV Therapy</td>
<td>Does partial DOT from trained treatment supporters improve immunologic and virologic outcomes in patients initiating antiretroviral therapy?</td>
<td>Single-site randomized controlled trial: 48-week partial DOT intervention vs standard of care (treatment supporter but no DOT) ( (n = 274) ) Cape Town, South Africa</td>
<td>Trial stopped 6 months early by DSMB because of futility No difference in proportion of patients with plasma HIV-1 RNA levels ( &lt; 50 ) copies/mL (primary endpoint) at 24, 48, 72, and 96 weeks However, odds ratio for death at 48 weeks in intervention arm was 0.3 (( P = .05 )) compared with control arm</td>
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<tr>
<td>Abstract 97.</td>
<td>Mortality and Virologic Outcomes of 2105 HIV-Infected Children Receiving ART in Soweto, South Africa</td>
<td>What are the clinical, immunologic, and virologic responses to antiretroviral therapy among children in South Africa?</td>
<td>Single-site observational cohort study ( (n = 2102) ) Soweto, South Africa</td>
<td>Plasma HIV-1 RNA at 18 months: 90% &lt; 400 copies/mL CD4+ percentage: baseline median, 11.5% 12-month mean, 25% 42-month mean, 31.7% Mortality rates: first 90 days, 14.4/100 child-years after 90 days, 1.99/100 child-years Loss to follow-up: 18% of children accessing care at least once 6% of those initiating antiretroviral therapy</td>
</tr>
<tr>
<td>Abstract 608.</td>
<td>Switching to Second-line ART, and Mortality in Resource-Limited Settings: Collaborative Analysis of Treatment Programs in Africa, Asia, and Latin America</td>
<td>What is the rate of switching from initial to second-line antiretroviral regimens in resource-limited settings and what factors predict this switch?</td>
<td>Multisite observational cohort study ( (n = 20,113) ) 17 programs in Africa, South America, and Asia ART-LINC</td>
<td>Change to a second-line regimen (primary outcome): 576 patients at median 22 months (IQR, 14–22); rate, 2.4/100 person-years Predictors of switch: low baseline CD4+ count; earlier switch associated with presence of plasma HIV-1 RNA monitoring at site Mortality (deaths/100 person-years): patients remaining on initial ART after immunologic or virologic failure, 10.7 patients switching to second-line ART, 5.1 patients on nonfailing initial regimens, 2.9</td>
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ART indicates antiretroviral therapy; ART-LINC, Antiretroviral Therapy in Lower Income Countries Collaboration; DOT, directly observed therapy; DSMB, Data and Safety Monitoring Board; IQR, interquartile range; OCTANE, Optimal Combination Therapy After Nevirapine Exposure; PMTCT, prevention of mother-to-child transmission.
with a thoughtful review of the epidemiology, clinical implications, and biology of transmitted drug resistance in resource-rich settings (Abstract 123). There is strong evidence that transmitted antiretroviral resistance compromises initial therapy and contributes to initial therapy failures. Recent cases illustrate the concerning clinical consequences of transmitted drug resistance. At present, however, large-cohort data from the CASCADE (Concerted Action on Seroconversion to AIDS and Death in Europe) study do not suggest that drug-resistant virus transmission results in an accelerated decline in CD4+ counts or clinical progression. In fact, drug resistance in general does not appear to be associated with progression, per an ALLRT (ACTG [AIDS Clinical Trials Group] Longitudinal Linked Randomized Trials) cohort case-control analysis (Abstract 659). Transmitted drug resistance is seen at various rates depending on the population and risk factors, which can make overall estimates of transmitted drug resistance difficult. Notably, there has been a rise in NNRTI resistance since 2000, reflecting treatment patterns. Most current guidelines recommend resistance testing at HIV diagnosis.

Transmitted drug resistance can be distinguished from resistance that emerges during drug treatment in that the infecting resistant virus undergoes rapid clonal expansion. Later, reversion to wild-type virus may occur because of fitness costs. Time to reversion of transmitted drug-resistant virus varies based on the mutations present. In an effort to detect small populations of mutant viruses, many investigators are using assays to detect low-frequency populations.

Transmitted drug resistance is likely underestimated. Metzner and colleagues analyzed baseline plasma samples from 93 acutely infected patients using the sensitive allele-specific real-time polymerase chain reaction (AS-PCR) to detect minority quasispecies (Abstract 649). None of the patients had resistance mutations detected by population sequencing, however, AS-PCR revealed the presence of the mutations M184V (12.1%) and K103N (6.5%). In this study, viruses were suppressed by a ritonavir-boosted PI regimen. Similarly, other investigators have found that in patients with detectable resistance mutations, using bulk sequencing, additional mutations can be revealed using assays for minority species. Numerous assays to detect minority populations are available that differ in their sensitivity and specificity.

Interpretation of the clinical and biologic meaning of results generated by these sensitive assays is crucial. Ideally, use of these assays would help guide initial treatment choices. Unfortunately, detecting most low-proportion minority subpopulations of transmitted drug resistance requires sampling within a short time of the primary infection (prior to reversion). The findings with regard to the minority species need to be understood within the context of the populations in which they are occurring as well as the length of time from the primary infection, which is rarely known.

The clinical implication of resistant minority populations was appreciated in ACTG 5095, which showed that the response to efavirenz-containing antiretroviral regimens was substantially reduced in patients with baseline resistance to NNRTI (ie, the mutation Y181C) detected by AS-PCR alone. Application of these findings in clinical management or epidemiology, however, remains unclear.

San Francisco cohort. Jain and colleagues delineated the increasing prevalence of NNRTI-associated drug resistance mutations in patients with acute or early HIV infection in San Francisco (Abstract 673). Two hundred twenty-four participants were enrolled in the observational cohort of patients with acute or early HIV infection who had yearly resistance test results from 2003 to 2007 available. Of those patients, 16% had evidence of transmitted drug resistance with the initial genotypic testing. The prevalence of NNRTI resistance–associated mutations was statistically significantly increased over time, whereas PI resistance remained stable in this population. The authors suggested that these findings may be attributable to the increasing use of NNRTI-based therapy.

Seattle cohort. Stekler and colleagues reported the prevalence of low-level mutations in primary HIV-1 infection and its effect on antiretroviral therapy in patients of the University of Washington Primary Infection Clinic (Abstract 674). One hundred patients had results from consensus sequencing and the oligonucleotide ligation assay to assess for low-frequency mutations at a median of 30 days from the estimated time of infection. Consensus sequencing detected mutations in 6 patients, and oligonucleotide ligation assay detected mutations in 28 additional patients. Among patients receiving antiretroviral therapy, no association was found between the presence of low-level mutations and time to virologic suppression. The authors posited that this surprising finding may be attributable to the small sample size as well as the increased potency of current antiretroviral therapies. However, it is also possible that the low-level mutations were false-positive results.

New York City. Castor and colleagues investigated the prevalence of transmitted drug resistance and phylogenetic clustering in a cohort of patients identified during acute and early HIV-1 infection in New York City (Abstract 500). Five hundred forty recently infected patients were enrolled from 1995 to 2008; data were grouped for the period from 1995 through 1999 and biennially for 2000 through 2008. Investigators found a prevalence of transmitted drug resistance of 19% in the cohort overall, which increased over time. In the 2007 to 2008 period, the prevalence was 23%. Specifically, the K103N mutation increased in prevalence from 1.9% to 6.7% from 2007 to 2008.

Young men of color. Hightow-Weidman and colleagues reported on transmitted drug resistance among young men of color who have sex with men (Abstract 905). Eighty-six men, aged 13 to 24 years, were enrolled in a prospective multicenter cohort who had baseline resistance testing before antiretroviral
therapy initiation. Most patients were new to care (84.9% in care < 3 months), African American (84.9%), and between 19 years and 22 years old (61.9%). Over 21% had a plasma HIV RNA level above 100,000 copies/mL at pretreatment. There was major resistance in 15 (17.4%) participants, of whom 9 had NNRTI resistance mutations, 4 had nRTI resistance mutations, 3 had the PI resistance mutation L90M, and 1 had cross-class resistance to nRTIs and NNRTIs. The authors urged early detection and secondary prevention efforts in young men of color.

**Low-Frequency Mutant Viral Subpopulations**

**Review of pyrosequencing** New techniques provide lower cost, complex sequence information on the HIV viral genome that include sequences of low-frequency populations that are not usually detected via standard sequencing techniques. High-throughput sequencing technologies parallelize the sequencing process, allowing for the processing of millions of sequences at once at lower cost per sequence. Known as pyrosequencing, this method relies on the light emission of pyrophosphates and has been commercialized. The ability to identify and sequence low-frequency subpopulations of virus is known as deep or ultradeep sequencing.

**Deep sequencing at time of virologic failure.** Le and colleagues discussed low-frequency HIV-1 drug-resistant variants from antiretroviral-experienced patients at the time of virologic failure (Abstract 684). Plasma samples from 22 antiretroviral-experienced patients with virologic failure were evaluated using ultradeep pyrosequencing compared with standard bulk sequencing. Low-frequency drug mutations made up 57% of the total 247 drug resistance mutations detected by deep sequencing, the majority (95%) of which were not detected by standard sequencing methods. Roughly 4 additional mutations were detected, on average, by ultradeep sequencing compared with standard sequencing. Of the 22 patients, 17 (77%) had additional low-abundance drug resistance mutations that increased the person’s level of resistance to 1 or more antiretroviral drug(s). Interestingly, thymidine analogue-associated mutations (TAMs) were detectable even in patients in whom a zidovudine- or stavudine-containing regimen was not failing. Nine of 10 patients had detectable TAMs despite having stopped treatment with thymidine analogues from 2 years to more than 7 years previously. These findings suggest that low-abundance HIV resistance mutations detected at the time of failure may partly reflect archived virus from virologic failure to prior regimens rather than to only the current failing regimen.

**Deep sequencing at reinitiation of antiretroviral therapy.** Swenson and colleagues presented a case series looking at the role of deep sequencing upon reinitiation of antiretroviral therapy in 7 antiretroviral-experienced patients with known resistance to prior regimens (Abstract 683). All 7 exhibited reversion to wild-type virus by standard genotypic testing up to the time of reinitiation of antiretroviral therapy. Deep sequencing was performed pretreatment and an average of 3 times during antiretroviral therapy until the virus became undetectable. Minority M184V, M184I, or both mutations were detected in 6 of the 7 baseline samples. These minority species neither reliably increased nor decreased in prevalence after the patients restarted lamivudine-containing regimens. In cases exhibiting changes in the predominance of M184V or M184I, the subpopulations remained rare at less than 1% of the virus population.

**Deep sequencing of integrase.** Ceccherini-Silberstein and colleagues compared standard sequencing with deep sequencing of integrase at baseline and at the time of treatment failure in patients receiving a raltegravir-containing regimen (Abstract 682). Standard sequencing did not reveal any major integrase mutations in 74 patients before initiation of a raltegravir-containing regimen. The investigators conducted deep sequencing on plasma samples from 6 patients, of whom 4 had experienced virologic failure and 2 had achieved virologic success. At baseline, quasispecies with substitutions at known integrase resistance positions were detected in the 4 participants experiencing virologic failure but not in the 2 with virologic success. The frequencies of these quasispecies were often close to the assay reliability limit. Quasispecies present at baseline were not necessarily associated with mutations at the same position from samples obtained during virologic failure. However, the authors suggested that baseline variability at known resistance positions may influence the antiviral efficacy of raltegravir, and they urge further investigation of such unrecognized complex pathways.

Liu and colleagues also reported on low-frequency mutations in integrase before initiation of a raltegravir-containing regimen and found that low-frequency mutations associated with raltegravir resistance were uncommon (Abstract 685). Of the 52 patients for whom raltegravir failed, 10 had evidence of integrase inhibitor resistance–associated mutations Q148K, Q148H, and Q140S at low frequencies (< 0.5% of genomes) and of L74M (14.3% of genomes). Pretreatment mutations in the principal pathways for resistance were uncommon and, when identified, were present at low frequencies. No statistically significant differences in pretreatment drug resistance mutations were noted between the treatment-failure and treatment-success groups.

**Viral tropism.** The use of deep sequencing for identification of viral tropism was also presented during a symposium, a themed discussion, and in numerous posters. See the CC Chemokine Receptor 5 Antagonists section below.

**Resistance to Nonnucleoside Reverse Transcriptase Inhibitors**

**Etravirine.** Marcelin and colleagues described the analysis of 243 treatment-experienced patients receiving an etravirine-containing regimen. Patients were exposed to a median of 6 nRTIs, 1 NNRTI, and 5 PIs at baseline.
(Abstract 645). Overall, 81.9% of patients achieved a virologic response, defined as a decrease of at least 1.5 log₁₀ copies/mL or a plasma HIV RNA level below 50 copies/mL at 2 months. Factors associated with virologic response were the number of new drugs used with etravirine (P < .0001) and the use of raltegravir, darunavir, or enfuvirtide for the first time. A history of exposure to efavirenz or nevirapine was associated with a poorer response (77% versus 91%, respectively; P < .03). Consistent with prior studies, the presence of K103N had no effect on the virologic outcome.

**Nevirapine and A376S.** The A376S mutation in the reverse transcriptase connection domain was associated with an increased risk of virologic failure of nevirapine-based therapy in NNRTI-naïve, HIV-infected subjects in the EuroSIDA study (Abstract 646). Paredes and colleagues evaluated the relationship of bulk genotypic sequencing before initiation of the NNRTI-containing regimen with virologic outcome in 287 NNRTI-naïve patients who were treated with nevirapine-based (n = 115) or efavirenz-based (n = 172) 3-drug regimens. Virologic failure was identified in 142 (49%) patients (77 receiving nevirapine and 65, efavirenz). The A376S mutation was associated with a greater than 10-fold increased risk of virologic failure with the nevirapine-based antiretroviral therapy but did not have an impact on the response to the efavirenz-based regimens.

**Resistance to Nucleoside Reverse Transcriptase Inhibitors**

**Zidovudine and tenofovir resistance.** Das and colleagues elucidated the structural basis for HIV-1 reverse transcriptase drug resistance to zidovudine and tenofovir (Abstract 67). X-ray crystallography was used to study the structural basis for the mechanisms of resistance to zidovudine and tenofovir. Investigators created crystal structures of reverse transcriptase of zidovudine-resistant HIV-1 and reverse transcriptase with the K65R mutation in various complexes, enhancing the understanding of the effects of these mutations on reverse transcriptase polymerization and excision, and on zidovudine and tenofovir resistance. They also investigated the effects of the presence of K65R in combination with other mutations and the clinical benefit of administering tenofovir along with zidovudine, emtricitabine, or lamivudine.

Radzio and colleagues presented biochemical and cell-based analyses of the N348I mutation (Abstract 68). The success of regimens containing zidovudine and nevirapine partly stems from the antagonistic interactions between TAMs and Y181C. The investigators hypothesized that N348I emerges to counteract this antagonism. This mutation occurs in the connection domain of HIV-1 reverse transcriptase in patients treated with these antiretroviral regimens. They found that the addition of Y181C to molecular clones of HIV-1 that included K70R or M41IT215Y mutations restored viral susceptibility to zidovudine. However, viruses containing Y181C, TAMs, and N348I displayed substantial levels of zidovudine resistance. This represents experimental evidence that N348I restores the zidovudine resistance phenotype in the presence of an antagonistic mutation, Y181C. The authors suggested that N348I may represent a novel pathway to dual zidovudine-NNRTI resistance.

Two presentations suggested that emtricitabine with tenofovir conferred more favorable resistance patterns than lamivudine with tenofovir does (Abstracts 642, 644).

Joyce and colleagues showed data on the risk of K65R mutations in viral populations after repeated interruptions of a regimen of zidovudine/lamivudine plus tenofovir in the DART (Development of Antiretroviral Therapy in Africa) trial (Abstract 681). The authors examined the presence of resistance using standard sequencing and pyrosequencing techniques for K65R and M184V in 18 patients treated with this antiretroviral regimen who underwent 4 successive structured treatment interruptions consisting of 12 weeks on and 12 weeks off therapy. All drugs were stopped together, and plasma HIV RNA level was measured 8 weeks into the cycles. There were 82 samples, of which 69 were taken when patients were off treatment. A single M184V mutation was detected in 1 patient, but no K65R mutations were detected in any sample by either method of viral sequencing. The authors concluded that, despite tenofovir’s longer half-life, the clinical risk of the emergence of tenofovir-associated mutations after treatment interruptions is low, and they note that this information may be useful in areas where intermittent drug shortages are common or in the context of PMTCT.

**Resistance to Protease Inhibitors**

**Substrate envelope hypothesis.** Nalam and colleagues described a process, based on the “substrate envelope hypothesis,” for designing PIs that avoid emergence of drug-resistant virus (Abstract 65LB). Current PIs bind the HIV-1 substrate envelope in a similar way, interfering with the protease function. Only a limited portion of the protease envelope is conserved, leaving a substantial portion at risk for the emergence of mutations leading to PI resistance in patients undergoing drug therapy. The team used computational designs to predict inhibitors that would fit within the confines of the substrate envelope, followed by synthetic chemistry to create the inhibitors and test the inhibitory function in wild-type and resistant HIV-1 variants. Crystal structures were determined, and the inhibitors were tested using a commercial phenotypic resistance assay (PhenoSense, Monogram Biosciences, South San Francisco, CA). The scaffolds for design were amprenavir and darunavir, which have high affinity for the substrate envelope.

From that point, thousands of inhibitors were designed after small adjustments were made in the scaffolds. Of those, 36 inhibitors were found to have very tight binding in the substrate envelope even in highly resistant viruses. Investigators tested 40 inhibitors against 3 viral subtypes by phenotypic testing. They found a series of 10 inhibitors that had higher affinity for HIV-1 protease than darunavir and many more that had higher affinity than lopi-
navir. Ten inhibitors had excellent affinity and success against wild-type and resistant viruses by phenotypic testing. The authors speculated that such techniques can be used for other HIV drug resistance targets and other diseases in which resistance emerges.

**Integrase Inhibitors**

**Review of raltegravir resistance.** Patterns of resistance in the integrase inhibitor class were a major focus at the conference. Raltegravir was approved in 2007 by the US Food and Drug Administration for use in treatment-experienced patients. Miller and colleagues shared analysis of resistance data from phase II and III clinical trials of raltegravir, emphasizing mutations and resistance pathways and implications for treatment-experienced and -naive patients (Abstract 125). Three major pathways to resistance have been identified: major mutations at sites Y143C/H/R, Q148H/K/R (preferred), or N155H. Minor mutations are also seen that lead to higher resistance. If none of these mutations is present at the time of virologic failure, they will likely emerge over time.

The impact of polymorphisms and minority variants on clinical response was explored. Integrase, like all HIV-1 proteins, is polymorphic. Baseline polymorphisms have little effect on susceptibility in vitro. Analysis of polymorphisms and clinical outcomes suggests that some polymorphisms may be associated with treatment outcome. In the BENCHMRK study (Blocking Integrase in Treatment-Experienced Patients with a Novel Compound Against HIV, Merck)-1 trial, at week 48, 51 patients experienced virologic failure, of whom 35 had raltegravir resistance; 138 patients were treatment responders. In an effort to identify polymorphisms associated with virologic failure, the investigators compared baseline polymorphisms in the 2 groups. No major mutations were identified at baseline. Minor polymorphisms were also uncommon (4 in the darunavir/r group; 7 in the lopinavir/r group), as was development of nRTI-associated resistance mutations (2 in the darunavir/r group; 5 in the lopinavir/r group). In both groups, a statistically significant proportion of patients meeting criteria for virologic failure had undetectable HIV RNA at follow-up visits without any change in regimen (30% in the darunavir/r group; 27.1% in the lopinavir/r group).

**Deep sequencing of integrase.** Again, the question of the utility of minority subpopulations was addressed for raltegravir. Liu and colleagues used parallel allele-specific sequencing technology to quantify minority raltegravir resistance–associated variants in patients participating in BENCHMRK-2, comparing the presence of baseline minority variants with virologic outcome and patterns of resistance (Abstract 685, also discussed above under Low-Frequency Mutant Viral Subpopulations).

**Raltegravir monotherapy.** Raltegravir in treatment-naive patients has been studied in a 2-phase treatment trial (Abstract 125). The first phase was raltegravir monotherapy compared with placebo for 10 days. Treatment-naive patients from this study were invited to participate in a noninferiority study comparing raltegravir at 3 different doses with efavirenz (all with tenofovir and lamivudine). Ninety-six-week follow-up of this study showed that raltegravir was noninferior to efavirenz (83% of patients receiving raltegravir and 84% of patients receiving efavirenz achieved plasma HIV RNA levels below 50 copies/mL).

Ultradeep sequencing was used to analyze the emergence of mutations associated with low-level raltegravir resistance in patients treated with raltegravir monotherapy followed by combination therapy (Abstract 125). Deep sequencing was performed at baseline, while receiving monotherapy, and just before the initiation of combination therapy. Resistance by ultradeep sequencing was identified infrequently and at very low levels and was not associated with virologic failure. At the end of 96 weeks of combination therapy, participants who received raltegravir monotherapy were not more likely to experience virologic failure than participants who initiated raltegravir as part of combination therapy. The sole monotherapy patient who experienced virologic failure through 96 weeks did not show any raltegravir resistance.

**Integrase resistance and fitness.** Fransen and colleagues reported that HIV-1 mutations at positions 143, 148, and 155 in integrase define different genetic barriers to raltegravir resistance in vivo (Abstract 69). Ninety-three subjects for whom raltegravir therapy was failing were included in the analysis. Clonal analysis, susceptibility, and replication capacity were assessed. Though viruses...
McCarthy and colleagues found that a combination of gp120 sequencing treatment-naive patients (Abstract notypic testing, with the Trofile assay in with HIV RNA levels below 10,000 copies/mL; populations of HIV-1 by the Trofile assay (Abstract 680). They found that 18% of the 202 samples had X4 virus use of less than 10%. The samples were from a clinical trial of a CCR5 antagonist, maraviroc, in patients with D/M virus. Patients harboring HIV-1 with less than 10% use of X4 had greater viral load declines with a maraviroc treatment than did either placebo recipients or patients with HIV-1 populations with greater than 10% X4 use. This suggests that CCR5 antagonists may be used in patients with D/M virus with a relatively low frequency of X4 use. Archer and colleagues used pyrosequencing to analyze samples from 3 patients with R5 virus detected by the Trofile assay prior to initiation of maraviroc treatment but who demonstrated emergence of D/M virus with maraviroc treatment (Abstract 679). In all 3 patients, ultradeep sequencing detected X4 virus at low frequencies (0.5%, 1.5%, and 6%) prior to initiation of maraviroc treatment and higher frequencies after treatment (81%, 99.9%, and 82%).

### Treatment of Acute Infection

The clinical and immunologic benefits of treatment during acute HIV infection are controversial. Nonrandomized studies have shown conflicting results. Steingrover and colleagues presented an analysis of the Dutch Primo-SHM embedded, acute-HIV-treatment cohort, comparing time off antiretroviral therapy in patients with primary HIV infection between 2003 and 2008 who were randomly assigned to no treatment or to treatment for 6 months or for 15 months (Abstract 70bLB). Of those enrolled, 102 were included in the analysis; 47 were untreated and 55 were treated. Pretreatment CD4+ count and viral load levels were similar in the 2 groups. Corrected Kaplan-Meier analysis, adjusted for time on therapy, showed that untreated patients started antiretroviral therapy after 126 weeks (95% CI, 104–150), whereas patients who initiated and then interrupted antiretroviral therapy remained off therapy for a mean of 181 weeks (95% CI, 161–201; \( P \ll 0.001 \)).

The French ANRS (National Agency for Research on AIDS and Viral Hepatitis) Primo Cohort investigators described those persons in the primary HIV infection cohort who, in the absence of treatment, spontaneously controlled the virus to plasma HIV RNA levels below 400 copies/mL for at least 12 months (Abstract 513). Of the 661 patients enrolled overall, 211 were untreated and 8 of these individuals spontaneously controlled viral replication. The median time from infection to HIV RNA level below 400 copies/mL was 7.0 months (range, 1.7–14.0). In 2 patients, viral control was lost at 4.0 years and 4.4 years after primary infection, and the remaining 6 patients maintained viral control until the last follow-up visit. Of the 8 controllers, none had viruses that harbored genotypic resistance, all had R5 virus, and 7 had subtype-B virus. Four of the 8 patients had HLA-B*57 or -B*27. Viral load during primary HIV infection was statistically significantly lower in controllers (3.0 log_{10} copies/mL) than in noncontrollers (4.7 log_{10} copies/mL; \( P \ll 0.001 \)), and controllers had statistically significantly higher CD4+ counts than noncontrollers during primary HIV infection.

### Outcomes From Treatment Cohorts in Non–Resource-Limited Settings

Two investigations applied novel analytic approaches to determine the optimal time to begin antiretroviral treatment using data from longitudinal cohorts of people living with HIV disease. In the first, Kitahata and colleagues took observational data from 60 geographic regions in the United States and Canada from the NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design) and tested the hypothesis
that initiation of antiretroviral therapy at CD4+ counts above 500 cells/µL improves survival (Abstract 71). Their analysis included 9155 study patients and 28,032 person-years of follow-up for HIV-infected individuals with CD4+ counts above 500 cells/µL who had active follow-up between 1996 and 2006. They excluded patients with prior antiretroviral therapy or AIDS-defining illnesses. The investigators attempted to mimic a randomized controlled trial by dividing participants into 2 groups, those who “deferred” treatment until CD4+ counts were below 500 cells/µL and those who initiated treatment with CD4+ counts above 500 cells/µL. Patients who did not initiate antiretroviral therapy within 1.5 years of reaching their target CD4+ count for antiretroviral therapy initiation were censored but not excluded. Inverse probability weights and adjusted Cox regression analysis allowed for control of time-varying, confounding, pretreatment differences between patients initiating or deferring treatment and for the informative censoring described above.

Within the cohorts, 2616 patients initiated antiretroviral therapy with CD4+ counts above 500 cells/µL, and 6539 deferred antiretroviral therapy. The percentage of patients initiating antiretroviral therapy with CD4+ counts above 500 cells/µL peaked in 1998 at 16% of the total cohort and decreased to less than 10% by 2003, reflecting standard-of-care treatment practices. Thus, the majority of patients in the early antiretroviral therapy subgroup received non–ritonavir-boosted PIs. Using inverse probability weighted Cox regression multivariate analysis, the relative hazard of death for those deferring antiretroviral therapy until their CD4+ count was below 500 cells/µL was 1.6 (95% CI, 1.3–1.9; P < .001) compared with those who initiated antiretroviral therapy with CD4+ counts above 500 cells/µL. These results were not altered by restriction of the inclusion criteria to patients with data on injection drug use and hepatitis C virus coinfection. They conducted a sensitivity analysis to demonstrate that an unmeasured confounder with a relative hazard of mortality of 4.0 would reduce the relative hazard of death for deferral only from 1.6 to 1.3 and concluded that their findings were robust.

Sterne and colleagues asked a similar question using data from the ART-CC (Antiretroviral Therapy Cohort Collaboration), a consortium of 15 cohorts from Europe and North America (Abstract 72LB). Prior data from this cohort collaboration showed increased rates of AIDS and death for patients initiating antiretroviral therapy with CD4+ counts below 250 cells/µL compared with rates in patients who initiated antiretroviral therapy with CD4+ counts above 350 cells/µL. The investigators extended these findings, using statistical techniques proposed by Cole and colleagues to estimate distributions of lead time and unreported events that are unmeasured in the ART-CC, impute lead time and unreported events for each individual deferring antiretroviral therapy to a lower CD4+ count range, and account for the error in these estimates with multiple imputation. The pretest antiretroviral therapy era data on 21,247 patients and 68,253 person-years of follow-up were derived from 7 observational cohorts that followed up patients between July 1989 and December 1995. The investigators restricted their dataset to non–injection-drug-using, AIDS-free individuals but did not control for other confounders.

Comparing data from patients initiating antiretroviral therapy with CD4+ counts of 351 cells/µL to 450 cells/µL with that of patients initiating at 451 cells/µL to 550 cells/µL, the HR for AIDS or death, adjusted for lead times and unreported events, was 0.99 (95% CI, 0.76–1.29). Comparing results from CD4+ count at initiation ranges of 0 cells/µL to 100 cells/µL with those of 101 cells/µL to 200 cells/µL showed a HR of 3.35 (95% CI, 2.99–3.75). Using this analysis for all 100-cell/µL ranges between 0 cells/µL and 500 cells/µL suggested that patients who defer antiretroviral therapy initiation to CD4+ count ranges below 350 cells/µL had an increased risk of AIDS or death. Similar comparisons for all-cause mortality alone did not show clear evidence that deferring antiretroviral therapy until CD4+ counts dropped below 250 cells/µL led to increased mortality rates. The investigators proposed that only a randomized, controlled trial could control for both measured and unmeasured confounders, but they concluded that delaying treatment until CD4+ counts are below 350 cells/µL confers an increased, through relatively small, absolute risk of AIDS and death.

Rosenblum and colleagues examined the relationship between antiretroviral therapy adherence, virologic failure, and the duration of continuous viral suppression among participants in the REACH (Reaching for Excellence in Adolescent Care and Health) cohort of HIV-infected homeless and marginally housed individuals receiving antiretroviral therapy in San Francisco (Abstract 583). They included 221 participants receiving antiretroviral therapy who achieved plasma HIV-1 RNA levels below 50 copies/mL and agreed to monthly, unannounced pill counts. Using a marginal structural model that controlled for nadir CD4+ count, regimen characteristics, past adherence, age, sex, depression, ethnicity, and drug or alcohol use, the investigators compared the probability of virologic failure immediately after achievement of virologic suppression to that after 12 consecutive months of virologic suppression. There was a statistically significant decrease in the risk of failure between these 2 time points for all individuals with at least 50% adherence to antiretroviral therapy. The estimated decrease in risk of failure was 0.39 (95% CI, 0.29–0.52) for those 90% to 100% adherent, 0.25 (95% CI, 0.05–0.50) for 75% to 89% adherence, and 0.45 (95% CI, 0.28–0.70) for 50% to 74% adherence. The authors concluded that the level of antiretroviral therapy adherence, correlating with the level of drug exposure necessary to maintain virologic suppression, decreases with increasing duration of virologic control but note that they cannot rule out selection bias in this observational study.

Lodwick and colleagues compared the risk of triple-class virologic failure among patients initiating either PI- or NNRTI-based regimens observed in 28 European longitudinal cohorts as a part of COHERE (Collaboration of Observa-
tional HIV Epidemiological Research Europe) (Abstract 585). The analysis was restricted to patients who were 16 years old or older, were followed up for at least 4 months, and initiated therapy with 2 nRTIs and either a PI or NNRTI. The authors defined virologic failure of an antiretroviral agent as occurring in individuals with a plasma HIV-1 RNA level above 500 copies/mL after at least 4 months of continuous use of that agent. Triple-class virologic failure was defined as failure of at least 2 nRTIs, 1 NNRTI, and a ritonavir-boosted PI. Overall, 45,937 people met inclusion criteria and started an initial antiretroviral regimen, 64% of whom received an NNRTI-based regimen. Of these, 980 (2.1%) experienced triple-class virologic failure, with the cumulative proportion of patients developing triple-class virologic failure rising from 3.4% (95% CI, 3.1–3.6) at 5 years from the start of antiretroviral therapy to 8.6% (95% CI, 7.5–9.8) at 9 years from antiretroviral therapy start. Using data from 2042 people who initiated PI-based regimens after the failure of NNRTI-based regimens, the investigators found that the rate of subsequent triple-class virologic failure was higher in patients who had lower CD4+ counts and higher plasma HIV-1 RNA levels, and lower for patients who spent less than 3 months taking antiretroviral therapy and had a plasma HIV-1 RNA level above 500 copies/mL. The authors noted that the rate of virologic failure did not decrease over time, which has implications for the need for future third-line treatment options in RLS.

**Pharmacokinetic Considerations**

**Antiretroviral Drugs in Pregnancy**

**Atazanavir/ritonavir and tenofovir.** Mirochnick and colleagues reported on atazanavir levels without and with tenofovir coadministration in pregnant women (Abstract 941). Women received atazanavir 300 mg with ritonavir 100 mg once daily; 13 were receiving concomitant tenofovir and 14 were not. The investigators found the area under the curve (AUC) and trough concentrations were reduced during the third trimester compared with those at 6 weeks to 12 weeks postpartum in both groups. The AUC and trough concentrations were lower in the group receiving tenofovir, as expected. The cord blood levels of tenofovir were approximately 20% that of maternal blood levels. The authors recommended increasing the dosage of atazanavir to 400 mg daily with ritonavir during pregnancy to assure adequate levels of the drug.

**Lopinavir/ritonavir.** Kiser and colleagues evaluated lopinavir/ritonavir pharmacokinetics in the second and third trimesters of pregnancy (Abstract 946). They made individual dose adjustments based on intensive pharmacokinetic sampling. They found that lopinavir and ritonavir were less protein bound during pregnancy. Eight of 10 women required a dose increase in the second trimester to achieve lopinavir concentrations consistent with historical control patients. Only 2 women required a further dose increase in the third trimester. This suggests that the dose of lopinavir/ritonavir should be increased in the second trimester, not the third trimester, as current guidelines advise.

**Nelfinavir.** Fang and colleagues presented data on nelfinavir in pregnancy (Abstract 943). They compared plasma levels of nelfinavir and M8, an active metabolite, in 16 pregnant, HIV-infected women in the second and third trimesters with those in women 6 weeks postpartum. The trough concentration of nelfinavir was 36% and 54% lower in the second and third trimesters, respectively, than concentrations in postpartum women. Trough M8 levels were undetectable in more than 50% of women during pregnancy. This suggests that, as in nonpregnant patients, nelfinavir is not the optimal PI for use in pregnancy.

**Antiretroviral therapy drugs in breast milk.** Investigators evaluated 8 mother-and-infant pairs in whom the mother was receiving zidovudine, lamivudine, and lopinavir/ritonavir (Abstract 947). Breast milk concentrations of zidovudine and lamivudine were 1.86 times and 5.6 times that of the mother’s plasma, respectively, whereas lopinavir and ritonavir concentrations were each 11% that of the plasma. No lopinavir, ritonavir, or zidovudine was detected in the infant’s plasma. A very low concentration of lamivudine, 1% that of the mother’s plasma, was detected in infants. The authors concluded that the low-to-undetectable antiretroviral drug concentrations found in the infants suggest that these drugs pose minimal risk of toxicity for infants. They also suggest that further studies should evaluate whether the low lopinavir/ritonavir concentration found in breast milk is associated with detectable HIV RNA in breast milk.

**Other Pharmacokinetic Considerations**

**Efavirenz in hair samples.** Gandhi and colleagues from the Women’s Interagency HIV Study presented data on efavirenz concentrations in hair samples (Abstract 692). Drug measurement in hair samples reflects the long-term exposure to a drug, unlike a single plasma sample, which measures very recent drug exposure. The authors found that efavirenz levels in hair samples were the strongest predictor of achieving virologic success on an efavirenz-based regimen. Self-reported adherence was not predictive. The authors suggested that this technique is a promising method to assess long-term exposure to antiretroviral drugs.

**Atazanavir and raltegravir.** Zhu and colleagues evaluated raltegravir monotherapy 400 mg twice daily, atazanavir monotherapy 300 mg twice daily, and the combination of atazanavir and raltegravir in 22 HIV-uninfected adults (Abstract 696). They noted that the raltegravir and atazanavir combination is a potential nRTI-sparing regimen that does not require ritonavir. Atazanavir trough concentrations were reduced by 29% when coadministered with raltegravir. However, all participants had trough concentrations above the accepted atazanavir target concentration of 150 ng/mL. Raltegravir trough concentrations were increased by 48% with coadministration. Electrocardiograms showed a QRS widening of 11 ms with atazanavir twice-daily therapy.
compared with pretreatment values. The clinical relevance of this effect is unknown.

**Very low dose ritonavir and saquinavir.** Van Der Lugt and colleagues examined saquinavir and ritonavir pharmacokinetics in 20 Thai HIV-infected patients (Abstract 697). All participants had virologic suppression with an antiretroviral regimen including saquinavir 1500 mg and ritonavir 100 mg given once daily. After a pretreatment pharmacokinetic assessment, patients received a reduced ritonavir dose of 50 mg and after 7 days underwent a second pharmacokinetic assessment. The authors found that ritonavir levels were statistically significantly lower after the dose adjustment but that saquinavir levels were not affected. These investigators used a liquid formulation of ritonavir and suggested that the strategy is not feasible in clinical practice until a more acceptable formulation reaches the market.

**Nevirapine and fluconazole.** Wakeham and colleagues compared nevirapine concentrations in 49 patients enrolled in a randomized, placebo-controlled trial of fluconazole for primary prophylaxis of cryptococcal disease (Abstract 700). The median nevirapine trough concentrations were increased in the presence of fluconazole, 5116 mg/mL in the treated group versus 3709 ng/mL in the placebo group ($P = .007$). However, no increase in hepatotoxicity among the larger study population was observed.

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A list of all cited abstracts appears on pages 89-95.

**Additional References**

