Advances in Antiretroviral Therapy

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The 17th Conference on Retroviruses and Opportunistic Infections maintained its tradition of being 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-naive and -experienced patients, the use of drugs for prevention of mother-to-child HIV-1 transmission, and HIV resistance to antiretroviral drugs. Of particular note were the continued advances in antiretroviral treatment and research emanating from resource-limited settings and from large clinical trials to determine the optimal initial antiretroviral drug regimen. Several interesting smaller studies were focused on HIV-1 pathogenesis and persistent viremia.

Investigational Drugs

Inhibitors of LEDGF/p75–Integrase Interaction

Christ and colleagues presented data on inhibitors of lens epithelium-derived growth factor p75 (LEDGF/p75) interaction with integrase (Abstract 49). They used the crystal structure of integrase to rationally design molecules to inhibit this interaction. In a series of experiments, they showed that these compounds inhibit HIV-1 integration, bind at a different pocket than strand transfer inhibitors, and are active against raltegravir-resistant isolates. The existing toxicity data are supportive of further drug development.

S/GSK1349572

Johns and colleagues presented data on the discovery and development of S/GSK1349572 (Abstract 55). This investigational drug is a once-daily integrase strand transfer inhibitor (INSTI) that retains antiviral activity in clinical isolates resistant to raltegravir and elvitegravir. Prior monotherapy studies have shown that the compound has potent antiviral activity through 14 days.[1]

QNL111

QNL111 is an integrase–DNA binding inhibitor whose antiviral activity was presented at the 2009 conference.[2] Thibaut and colleagues presented further data this year confirming the mechanism of action (Abstract 492). They found that QNL111 decreased the amount of integrated DNA in cell culture, similar to raltegravir, a strand transfer inhibitor. In contrast to raltegravir, QNL111 did not lead to an increase in 2-long-terminal-repeat (LTR) circles, consistent with inhibition of HIV-1 integration before the strand transfer reaction.

CC Chemokine Receptor 5 Antagonists

Cohen presented data on TBR-652, a CC chemokine receptor 5 (CCR5) antagonist (Palleja et al, Abstract 53). The drug is dosed once daily and has a half-life of approximately 40 hours. TBR-652 is also an antagonist of CC chemokine receptor 2 (CCR2), which is found on monocytes, immature dendritic cells, and memory T cells. This receptor has been associated with the pathogenesis of atherosclerosis and the metabolic syndrome. The antiviral activity, safety, tolerability, pharmacokinetics, and CCR2 activity were investigated in a randomized, double-blinded, placebo-controlled, dose-escalating study in HIV-infected patients. Eligible subjects were treatment experienced with no HIV treatment for at least 6 weeks who had CCR5-tropic HIV, a CD4+ cell count of 250 cells/μL or more, and a plasma HIV-1 RNA level of at least 5000 copies/mL. Forty-four subjects were given 25-mg to 150-mg doses, and 10 received placebo for 10 days without other antiretroviral medications. The maximal decline in plasma HIV-1 RNA level was 1.4 $\log_{10}$ copies/mL to 1.8 $\log_{10}$ copies/mL for dose levels above 25 mg. The pharmacokinetic analysis suggested dose-proportional increases in exposure through the range of doses tested and a half-life ranging from 23 hours to 48 hours. There were no serious adverse events and no obvious safety concerns. Monocyte chemotactic protein 1 (MCP-1), the native ligand for CCR2, increased during dosing of TBR-652 relative to placebo. This suggests that TBR-652 was blocking CCR2 in these subjects. A detailed pharmacokinetic analysis was presented in Abstract 598.

Genetically Modified CD4+ T cells

There were 2 studies that evaluated genetically modified T cells as a therapeutic strategy. The first used zinc-finger nucleases to knock out the CCR5 gene in human CD34+ hematopoietic stem cells (Abstract 387). These stem cells were infused into a mouse model, and the mice were challenged with HIV-1. CCR5-deleted cells were rapidly selected, and viral replication was controlled by 12 weeks after infection.

In the second study, Tebas and colleagues reported results from an ongo-

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ing clinical trial of autologous CD4+ T cells genetically modified with a lentiviral vector to express long antisense to HIV *env* (Abstract 388). Subjects were taking suppressive antiretroviral therapy and received 3 to 6 infusions of 10^{10} modified autologous CD4+ T cells. They underwent structured treatment interruptions after the last infusion. Of the 13 patients who interrupted treatment, 8 were evaluable for the primary endpoint. Seven of 8 had a reduction in the viral load setpoint (range, decrease of 0.26 – 0.98 $\log_{10}$ copies/mL), and 1 subject had not had detectable virus at 104 days postinterruption along with an increase in CD4+ T-cell count.

Banana Lectin

Swanson and coworkers presented data on banana lectin (Abstract 487). This compound exhibited low nanomolar or picomolar inhibition of HIV-1 replication. Quantification of reverse transcriptase products suggests that banana lectin blocks HIV-1 entry similar to other mannose-specific lectins. This compound can also bind gp120, and the authors suggested that this compound may be considered for development as a microbicide.

Inhibitor of Reverse Transcriptase Dimerization

HIV reverse transcriptase is a heterodimer of p51 and p66. Agopian and colleagues presented data on peptide inhibitors of reverse transcriptase dimerization (Abstract 494). They constructed 2 peptides that exhibited low nanomolar inhibitory concentrations when delivered as nanoparticles to a cell culture system. These peptides showed activity against a broad range of HIV-1 subtypes and resistant isolates.

Immune-Based Therapies

Therapeutic HIV-1 *gag* Vaccine

Li and colleagues presented additional data from ACTG (AIDS Clinical Trials Group) A5197, a randomized, placebo-controlled trial of therapeutic vaccination with an adenovirus 5 HIV-1 *gag* vaccine for reduction of the viral load setpoint after treatment interruption (Abstract 76). Participants with stable virologic suppression with antiretroviral therapy received vaccine or placebo injections over 6 months, followed by treatment interruption for at least 16 weeks. In this analysis, investigators evaluated host and viral factors associated with viral rebound after treatment interruption. They classified participants according to HLA typing as having unfavorable, neutral, or protective alleles. There was no vaccine effect in subjects with unfavorable or protective alleles. Investigators did observe a lower plasma HIV-1 RNA level 16 weeks after treatment interruption for participants with neutral alleles who received vaccine compared with placebo (4.0 vs 4.6 $\log_{10}$ copies/mL; P = .01). Factors associated with lower plasma HIV-1 RNA levels after treatment interruption included absence of unfavorable HLA alleles, greater divergence of *gag* mutations for the vaccine sequence, lower plasma HIV RNA level before antiretroviral therapy initiation, and randomization to the vaccine arm. The authors noted that HLA determination and pre–antiretroviral therapy plasma HIV RNA levels should be considered in future therapeutic vaccine studies.

Autologous Dendritic Cell Vaccine

Plana and colleagues presented the results of a phase I, double-blind, placebo-controlled trial of a therapeutic vaccine using autologous dendritic cells pulsed with a high dose of autologous HIV-1 (Abstract 77). Eligible subjects were not receiving antiretroviral therapy and had a CD4+ cell count above 450/µL and a plasma HIV-1 RNA level above 1000 copies/mL. Subjects received dendritic cells pulsed with HIV or a placebo of dendritic cells without pulsing at entry, week 2, and week 4. The treatments appeared safe with no substantive local reactions or any evidence of autoimmunity. There was a modest difference in plasma HIV-1 RNA level between groups at weeks 24 and 48 (.20 and .31 copies/mL reductions in vaccine recipients compared with .21 and .34 copies/mL increases in placebo recipients; P = .03 and P = 0.05, respectively). There was no difference in CD4+ cell counts between groups. Vaccine recipients showed a statistically significant negative correlation between changes in plasma HIV-1 RNA level and HIV-specific T-cell responses compared with placebo recipients, in whom a positive correlation was observed.

Clinical Trials of Antiretroviral Therapy in Treatment-Naive Patients

AIDS Clinical Trials Group A5202

ACTG A5202 was a randomized equivalence study in antiretroviral-naive patients that was a double-blind, placebo-controlled comparison of abacavir/lamivudine versus tenofovir/emtricitabine and an open-label comparison of atazanavir 300 mg/ritonavir 100 mg versus efavirenz 600 mg (Abstract 59LB). The inferior antiretroviral activity of abacavir/lamivudine versus tenofovir/emtricitabine in subjects with a screening plasma HIV-1 RNA level above 100,000 copies/mL has been reported.[3]

Daar, on behalf of the ACTG A5202 study team, presented the final results of this trial. The study enrolled approximately 1800 participants aged 16 years or older with a plasma HIV-1 RNA level above 1000 copies/mL. The baseline characteristics of the study population were as follows: 83% were men, median age was 38 years, the median plasma HIV-1 RNA level was 4.7 $\log_{10}$ copies/mL, and the median CD4+ cell count was 230/µL. Only 45% had genotypic testing at some point before study entry.

The primary efficacy endpoint was time to confirmed virologic failure (a composite of plasma HIV-1 RNA level ≥ 1000 copies/mL at week 16 or ≥ 200 copies/mL at week 24 or later). In those with a screening plasma HIV-1 RNA level below 100,000 copies/mL, there was no appreciable difference in the time to virologic failure between abacavir/lamivudine and tenofovir/emtricitabine when given with efavirenz

or atazanavir/ritonavir. There was no appreciable difference in the time to virologic failure for atazanavir/ritonavir versus efavirenz when given with abacavir/lamivudine or tenofovir/emtricitabine. None of these comparisons reached prespecified equivalency bounds for the hazard ratio (HR). The number of events was much smaller than hypothesized, leading to wider confidence intervals (CIs).

Abacavir/lamivudine was associated with a shorter time to grade 3 or grade 4 signs, symptoms, or laboratory abnormalities than was tenofovir/emtricitabine when given with efavirenz. The abacavir/lamivudine groups were associated with a shorter time to regimen change. This appeared to be wholly explained by abacavir hypersensitivity reaction. Of note, testing for the HLA-B*5701 allele was not routinely performed at the time this trial was enrolling participants.

The most notable difference between efavirenz and atazanavir/ritonavir was in the rate of the emergence of genotypic evidenced resistance. Almost no protease inhibitor (PI) resistance mutations emerged at virologic failure compared with the efavirenz-receiving groups, in whom failure was associated with nonnucleoside analogue reverse transcriptase inhibitor (NNRTI)–associated mutations.

Elvitegravir/Cobicistat/Tenofovir/Emtricitabine

Cohen and colleagues presented data from 2 clinical trials involving the investigational drug cobicistat (GS-9350), an inhibitor of cytochrome P3A4 (CYP3A4) without anti-HIV activity (Abstract 58LB). Both trials enrolled antiretroviral-naive subjects, without hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, and with CD4+ cell counts above 50/μL, plasma HIV-1 RNA levels above 5000 copies/mL, and no evidence of resistance through genotypic testing. The median age of participants in the treatment groups was between 34 and 37 years; 86% to 94% were male; 55% to 78% were white; median baseline CD4+ cell count was 341/μL to 436/μL, and the mean plasma HIV-1 RNA level was 4.6 $\log_{10}$ copies/mL to 4.7 $\log_{10}$ copies/mL. Both studies were randomized, double blind, and placebo controlled.

The first study assigned 48 subjects to elvitegravir 150 mg, an investigational INSTI, in a fixed-dose, once-daily formulation with tenofovir, emtricitabine, and cobicistat 150 mg, and it assigned 23 subjects to a fixed-dose combination of tenofovir, emtricitabine and efavirenz. At 24 weeks postrandomization, 90% of the elvitegravir and 83% of the efavirenz recipients achieved a plasma HIV-1 RNA level below 50 copies/mL in the intention-to-treat (ITT), missing = failure (M = F) analysis. The observed difference was 5% (95% CI, −11% to +21%), adjusting for differences in baseline plasma HIV-1 RNA level. There were no cases of virologic failure in either group. Adverse events related to randomized treatment were observed in 35% and 57%, respectively. Serious adverse events were rare, and only 1 subject receiving efavirenz discontinued treatment because of an adverse event.

The second trial assigned 50 subjects to cobicistat 150 mg and 29 subjects to ritonavir 100 mg (Abstract 58LB). Both drugs were given once daily with atazanavir 300 mg plus fixed-dose tenofovir/emtricitabine. In the ITT, M = F analysis, 84% and 86%, respectively, achieved plasma HIV RNA levels below 50 copies/mL. Only 1 subject experienced virologic failure. Adverse events related to randomized treatment were observed in 20% and 24%, respectively. Serious adverse events were rare; 2 (4%) subjects receiving cobicistat and 1 (3%) receiving ritonavir discontinued treatment because of an adverse event.

Cobicistat was associated with a decrease in the estimated glomerular filtration rate (eGFR) by the Cockcroft-Gault equation. The investigators evaluated this further in a small safety study in HIV-uninfected volunteers. The eGFR was reduced with cobicistat but the actual GFR was not affected. This result is consistent with inhibition of tubular secretion of creatinine and not a renal toxicity per se. The results were supportive for phase III studies of the elvitegravir/cobicistat fixed-dose pill and for phase III studies of cobicistat as an alternative to ritonavir.

Nevirapine Versus Lopinavir/Ritonavir

One randomized, controlled trial of initial antiretroviral therapy in resource-limited settings (RLS) was presented at this year's conference. McIntyre and colleagues presented data from the OCTANE-2 (Optimal Combination Therapy After Nevirapine 2) trial conducted in South Africa (Abstract 153LB). The OCTANE-2 trial was an open-label, equivalence study comparing the efficacy of nevirapine to lopinavir/ritonavir (/r), both coadministered with tenofovir/emtricitabine, in antiretroviral therapy–naive women in South Africa with no history of single-dose nevirapine exposure for prevention of mother-to-child transmission (PMTCT) of HIV. (This trial is a companion trial to OCTANE-1, in which identical regimens were compared in antiretroviral therapy–naive women with a history of exposure to single-dose nevirapine for PMTCT.[4] OCTANE-1 showed a higher rate of death and virologic failure in the nevirapine group than in the lopinavir/r group).

Five hundred antiretroviral therapy–naive women with no prior history of single-dose nevirapine were enrolled, with 250 women in each treatment group. Inclusion criteria included a CD4+ cell count below 200/μL and a creatinine clearance rate greater than 60 mL/min. Women with prior exposure to zidovudine as part of PMTCT were eligible to participate if exposed to zidovudine for less than 10 weeks and if the exposure occurred more than 6 months before enrollment. The primary endpoint was time to death or virologic failure. Virologic failure was defined as a confirmed plasma HIV RNA level less than 1 $\log_{10}$ below baseline at 12 weeks or a plasma HIV RNA level above 400 copies/mL at 24 weeks. Follow-up was for at least 48 weeks after last enrollment, which was completed in August 2009. The study was powered to assess equivalence, with a CI of 95% for the HR range of 0.5 to 2.0. An ITT analysis was performed.

Baseline characteristics were as follows: median age, 34 years; median CD4+ cell count at baseline, 121/μL; plasma HIV-1 RNA level, 5.15 $\log_{10}$ copies/mL; chronic HBV infection, 7%; prior exposure to zidovudine, 1%. One subject with prior exposure to single-dose nevirapine was enrolled in error and was randomly assigned to the lopinavir/r group. Of a random sample of 199 women for whom genotypic analysis was performed, nevirapine resistance-associated mutations (RAMs) were observed in 0.6%. Seventy-one percent of participants had subtype-C HIV. Overall, 4.0% were lost to follow-up (LTFU); it was twice as high in the nevirapine recipients.

The primary endpoint was reached by 92 women: 17% in the nevirapine group and 20% in the lopinavir/r group, yielding a HR of 0.82 for the primary endpoint in the nevirapine recipients compared with the lopinavir/r group. Thus, the study met the prespecified criteria for equivalence between groups. Overall, there were 5 deaths, 2 in the nevirapine group and 3 in the lopinavir/r group. The majority of endpoints reached were driven by virologic failure. The authors also presented data on the "time to permanent discontinuation of a regimen," a composite of death, virologic failure, LTFU, or adverse events. In this analysis, 28% of subjects in the nevirapine group, compared with 9% in the lopinavir/r group, permanently discontinued treatment. These discontinuations were driven primarily by adverse events in the nevirapine recipients. Although similar rates of adverse events were observed in the 2 study groups, 14% of the nevirapine discontinuations were due to adverse events, mainly rash, whereas no discontinuations in the lopinavir/r group were due to adverse events ($P < .0001$). The authors postulated that strict protocol criteria for adverse event–mandated discontinuations may have led to the high rate of discontinuation in the nevirapine group. In sum, and in contradistinction to the results of OCTANE-1, a nevirapine-based regimen was equivalent to lopinavir/r–based therapy with respect to virologic failure and death in antiretroviral therapy–naive women with no prior exposure to single-dose nevirapine.

Clinical Trials of Antiretroviral Therapy in Treatment-Experienced Patients

Phase III Trials of Vicriviroc

Gathe and colleagues presented the results of 2 identical phase III, randomized, placebo-controlled trials of the investigational CCR5 inhibitor vicriviroc in treatment-experienced subjects (Abstract 54LB). Eligible subjects had documented resistance to 2 or more drug classes (nucleoside analogue reverse transcriptase inhibitor [nRTI], NNRTI, or PI) or at least 6 months of antiretroviral therapy experience. Originally, subjects were required to have only R5 virus as determined by tropism testing, but the analysis described here includes those subjects confirmed to have only R5 virus on retesting of screening samples using an enhanced-sensitivity tropism assay. Subjects were randomly assigned 2:1 to vicriviroc 30 mg or placebo with an optimized background regimen containing 2 or more active antiretroviral drugs predicted to have activity based on resistance testing. The primary endpoint was the proportion with plasma HIV-1 RNA levels below 50 copies/mL 48 weeks after randomization.

The 2 trials included 375 and 346 randomized subjects, respectively. In a pooled analysis, the median age of subjects was 43 years; 60% were white; and 29% were female. The mean baseline plasma HIV-1 RNA level was 4.6 $\log_{10}$ copies/mL, the mean CD4+ cell count was 257/μL, and 61% of subjects had 3 or more active drugs in their background regimens.

Pooling results from the 2 trials, investigators found no difference in the proportion with plasma HIV-1 RNA levels below 50 copies/mL at week 48: 64% of the vicriviroc group versus 62% of the placebo group. There was no difference in the CD4+ cell count gain between groups. In a prespecified subgroup analysis of subjects with 2 or fewer active drugs in the background regimen, vicriviroc led to improved virologic suppression (70% vs 55%; $P = .02$). There were 71 participants receiving vicriviroc with protocol-defined virologic failure. Emergence of CXC chemokine receptor 4 (CXCR4) use was observed in 9 (13%), and 3 (4%) had vicriviroc resistance. There was no obvious difference in observed adverse events. There were no seizures in the vicriviroc group and no evidence of increased malignancy. The antiviral activity of the background regimens may have made it more difficult to detect an additional effect of vicriviroc.

Once- Versus Twice-Daily Darunavir/Ritonavir

Daruanvir has US Food and Drug Administration approval for once-daily dosing for treatment of HIV infection in antiretroviral–naive subjects. Cahn and colleagues presented data from a clinical trial comparing darunavir/r given 800 mg/100 mg once daily and 600 mg/100 mg twice daily in HIV-1-infected patients whose prior antiretroviral regimen was failing and who had no darunavir RAMs on genotypic testing (Abstract 57). All subjects received an optimized background regimen of 2 or more nRTIs. The 590 subjects underwent randomization. The treatment groups included 33% and 39% women and had a mean plasma HIV-1 RNA level of 4.1 $\log_{10}$ copies/mL and 4.2 $\log_{10}$ copies/mL and a median CD4+ cell count of 219/μL and 236/μL, respectively. Approximately 46% of subjects had no prior PI experience, and 85% of viruses were susceptible to all available PIs at baseline. The highest fold increase in darunavir susceptibility in any subject was 1.9.

The primary endpoint was the proportion of subjects with plasma HIV RNA level below 50 copies/mL at week 48. Once-daily dosing was noninferior to twice-daily dosing (72% vs 71%; 95% CI, −6% to 9%). There was no difference in response when stratifying by baseline plasma HIV-1 RNA level ($<$ 50,000 copies/mL and $\geq$ 50,000 copies/mL). The median trough darunavir concentrations in the once- and twice-daily groups were 1896 ng/mL and

3197 ng/mL, respectively. The once-daily group had lower elevations of triglyceride levels and total cholesterol levels than the twice-daily group.

Antiretroviral Treatment Strategies

Antiretroviral Therapy During Acute or Recent HIV-1 Infection

Hogan, speaking on behalf of the ACTG A5217 study team, investigated whether antiretroviral therapy given early in HIV-1 infection would preserve HIV-specific immune responses and lead to a lower viral load setpoint after antiretroviral therapy interruption (Abstract 134). Eligible subjects had recent (but not acute) HIV-1 infection, defined as positive for HIV-1 on enzyme-linked immunosorbent assay (ELISA) and Western blot testing, with a negative result either on ELISA or detuned ELISA or an indeterminate result on Western blot testing within the 6 months prior to randomization. Subjects were excluded if they had a CD4+ cell count below 350/µL or a plasma HIV-1 RNA level below 500 copies/mL. Subjects underwent randomization to either remain without antiretroviral therapy or to receive a 36-week course of antiretroviral therapy (lopinavir/r, tenofovir/emtricitabine) followed by treatment interruption. All subjects were observed for 96 weeks.

Of a planned sample size of 150, 130 were enrolled and had the following characteristics: 90% men; median CD4+ cell count of 540/µL; and median plasma HIV-1 RNA level of 4.4 $\log_{10}$ copies/mL. The primary endpoint was a plasma HIV-1 RNA level of 1 $\log_{10}$ copies/mL at week 72. The criteria for antiretroviral therapy initiation postrandomization were consistent with treatment guidelines at the time: primarily a CD4+ cell count below 350/µL. The Data and Safety Monitoring Board recommended early stoppage of the study based on the probability that the findings regarding the primary analysis presented below would persist and that no additional study goals would be achieved by continuing the study as currently designed. Investigators found that the mean viral load at week 72 of the treated group (36 weeks after antiretroviral therapy interruption) was lower than that of the untreated group at 72 or 36 weeks postrandomization (P = .005 and P = .002, respectively).

However, the authors noted that these data are difficult to interpret because many of the plasma HIV-1 RNA values in the untreated group were imputed due to the initiation of antiretroviral therapy before week 72. Indeed, among the 79 subjects with at least 72 weeks of follow-up at the time of analysis, 50% of the patients in the untreated group initiated antiretroviral therapy before week 72 compared with 10% reinitiating antiretroviral therapy in the group randomly assigned to initial treatment. The authors estimated that initial limited antiretroviral therapy during early HIV infection had delayed the need for subsequent antiretroviral therapy by approximately 16 weeks when excluding the initial 36-week course of antiretroviral therapy. The observed disease progression in the untreated group was much higher than expected and provides support for earlier initiation of antiretroviral therapy as currently recommended by major guidelines.

Raltegravir Intensification

Buzon and colleagues conducted a randomized, placebo-controlled trial in which raltegravir or placebo was added to a suppressive antiretroviral therapy regimen (Abstract 100LB). They randomly assigned 69 subjects receiving suppressive antiretroviral therapy to add raltegravir (n = 45) or placebo (n = 24) to their regimen for 48 weeks. No difference was found between groups in the amount of total HIV-1 DNA or integrated DNA in peripheral blood mononuclear cells (PBMCs) or in the plasma HIV-1 RNA level as measured by a single-copy assay. In the raltegravir group, 2-LTR circles increased 2 weeks and 4 weeks postinitiation and decreased thereafter. Theoretically, 2-LTR circles should increase during integrase strand transfer inhibition of active HIV-1 replication. The investigators did not find similar changes in the placebo group. In a post hoc analysis, subjects in the raltegravir group who had 2-LTR circles at some point during follow-up had higher baseline CD8+ cell activation that decreased with raltegravir. The authors concluded that this was evidence of ongoing productive replication in at least a subset of participants.

Two other single-arm studies examined the addition of raltegravir to a suppressive regimen. Yukl and colleagues reported on 7 subjects whose plasma, PBMC, and gut biopsy specimens (duodenum, ileum, colon, and rectum) were sampled before and after adding raltegravir (Abstract 279). Plasma HIV-1 RNA level, as measured by a modified ultrasensitive viral load assay, was not affected. The investigators did not find any definitive changes in HIV-1 DNA or RNA levels in the gut or PBMC specimens, except for a decrease of HIV-1 RNA in the ileum from 3438 copies to 682 copies per 10^6 CD4+ T cells. Interestingly, this decrease was associated with an increase in CD4+ T cells and a decrease in percent of CD38+HLA-DR+ in CD8+ T cells in the ileum, suggesting reduced virologic replication in this site. Weigand and colleagues enrolled 8 subjects with a history of prior virologic failure (mean of 4 prior failed regimens) who added raltegravir to their regimens for 4 weeks (Abstract 280). They found no effect on plasma HIV-1 RNA level as measured by the single-copy assay.

Addition of Enfuvirtide to an Initial Antiretroviral Regimen

Joly and colleagues compared CD4+ T-cell responses in those initiating antiretroviral therapy at a very low CD4+ cell count with or without the addition of enfuvirtide to a standard antiretroviral drug regimen (Abstract 282). CD4+ cell responses did not differ between groups, but more subjects receiving enfuvirtide had a plasma HIV-1 RNA level below 50 copies/mL 24 weeks postrandomization than did control subjects (74% vs 58%, respectively; P = .03). There did not appear to be appreciable differences in this parameter at 48 weeks.

Table 1. Selected Studies of Antiretroviral Therapy Intensification

Abstract No. Authors	Intensification Drug (sample size)	Duration of Intensification	Population	Design	Virologic Outcomes	Other Outcomes
Abstract 100LB Buzon et al	Raltegravir (n = 45) or placebo (n = 24)	48 weeks	HIV-1-infected adults with virologic suppression receiving antiretroviral therapy	Randomized, placebo-controlled trial	No difference between groups by HIV-1 single-copy assay	No difference in total HIV-1 DNA or integrated DNA in peripheral blood mononuclear cells or in plasma. 2-long-terminal-repeat circles increased in the raltegravir group at 2 weeks and 4 weeks postinitiation and decreased thereafter
Abstract 279 Yukl et al	Raltegravir (n = 7)	12 weeks	HIV-1-infected adults with virologic suppression receiving antiretroviral therapy	Single-arm, open-label trial for 12 weeks	No change in HIV-1 RNA or DNA in plasma, peripheral blood mononuclear cells, or gut biopsies except for decreased HIV-1 RNA level in the ileum	Change in HIV-1 RNA in the ileum was associated with locally decreased CD8+ cell activation
Abstract 280 Wiegand et al	Raltegravir (n = 8)	30 days	HIV-1-infected adults with current virologic suppression and history of prior virologic failure	Single-arm, open-label trial	No change in HIV-1 RNA observed in patients while receiving raltegravir, compared with periods before or after adding raltegravir	No change in CD4+ cell counts
Abstract 282 Joly et al	Enfuvirtide (n = 101) or control (n = 94)	48 weeks	HIV-1-infected adults with CD4+ cell count < 100/μL initiating antiretroviral therapy with and without enfuvirtide	Randomized, controlled, open-label trial	Higher rate of virologic suppression to < 50 copies/mL at week 24 with enfuvirtide (74% vs 58%; $P = .03$)	No difference in CD4+ cell responses between groups
Abstract 284 Gutierrez et al	Maraviroc (n = 9)	12 weeks	HIV-1-infected adults with virologic suppression, CD4+ cell count > 350/μL, R5 tropism prior to treatment	Single-arm, open-label trial	Increase in HIV-1 RNA level after 12 weeks by single-copy assay	Increase in 2-long-terminal-repeat circles, decrease in HLA-DR+/CD38+ in CD8+ cells
Abstract 285 Wilkin et al	Maraviroc (n = 32)	24 weeks	HIV-1-infected adults with current virologic suppression and poor immune response	Single-arm, open-label trial	Not assessed	Decrease in immune activation as assessed by CD38+, and HLA-DR+/CD38+ in CD4+ and CD8+ T cells

Maraviroc Intensification

Two studies examined the addition of maraviroc to a suppressive regimen. Gutierrez and colleagues enrolled 9 subjects with a CD4+ cell count above 350/μL, a prior sample showing CCR5-tropic virus, and suppressed plasma HIV-1 RNA level for at least 1 year (Abstract 284). The authors suggested that a decrease in the latent reservoir was seen: 6 of 9 patients had replication-competent virus detectable on at least 1 of 2 baseline measurements compared with 1 of 9 patients at week 12. An increase was seen in plasma HIV-1 RNA level as measured by a single-copy assay at week 12: 1 of 9 subjects had detectable virus at baseline compared with 6 of 7 subjects at week 12. An increase in 2-LTR circles was also shown: 0 of 9 subjects had HIV detected at baseline compared with 5 of 9 subjects at week 12. The percent of CD38+HLA-DR+ in CD8+ T cells at week 12 was decreased, suggesting decreased immune activation (5.4% vs 2.3%, respectively; $P = .08$).

Wilkin and colleagues presented results of ACTG A5256, a single-arm pilot trial of maraviroc intensification in patients with suboptimal immune response despite sustained virologic suppression (Abstract 285). They enrolled subjects with a CD4+ cell count below 250/μL, a calculated CD4+ slope of −20 cells/μL/year to +20 cells/μL/year, and an undetectable plasma HIV-1 RNA level for longer than 48 weeks before study entry. Subjects added maraviroc to their suppressive regimen for 24 weeks. The median increase in CD4+ cell count was 11/μL (90% CI, 1 – 22/μL). The lower bound did not exclude 20/μL, and the strategy was not considered successful. A statistically significant decrease in percent CD38+ and percent CD38+HLA-DR+ expression in both CD4+ and CD8+ T cells was noted, suggesting reduced immune activation. These studies of antiretroviral therapy intensification are summarized in Table 1.

Valproic Acid to Decrease Latent Reservoir

Valproic acid is an inhibitor of histone deacetylase in vitro. Persistence of latently infected CD4+ T cells is a major challenge for HIV eradication. The histone deacetylase inhibitors are of major interest to promote HIV transcription to render these infected cells susceptible to antiretroviral therapy. Routy and colleagues presented the results of a randomized, placebo-controlled, cross-over study of valproic acid (Abstract 496). The primary endpoint was a statistically significant reduction in the proportion of memory CD4+ T cells carrying HIV proviral DNA after 16 weeks of valproic acid or placebo. Eligible subjects were taking antiretroviral therapy for at least 12 months and had a plasma HIV-1 RNA level below 50 copies/mL and a CD4+ cell count of 200/μL or more. Fifty-six subjects were enrolled: 74% were men and the median CD4+ cell count was 537/μL. Twelve subjects did not complete the protocol. There was no difference in the primary endpoint between groups: 8 of 21 (38%) subjects taking valproic acid had a statistically significant reduction versus 7 of 18 (39%) subjects receiving placebo (P = .99). Similar results were found after the cross-over was completed.

Outcome Predictors in Resource-Rich Settings

Numerous abstracts evaluated predictors of clinical and immunologic outcomes in resource-rich settings (RRS). Sighem and colleagues presented data on rates of progression to death in HIV-infected patients who have not initiated antiretroviral therapy (Abstract 526). The study included 4612 patients from the ATHENA (AIDS Therapy Evaluation in the Netherlands) cohort who received a diagnosis of HIV infection between 1998 and 2007. The investigators compared mortality in HIV-infected patients who were antiretroviral therapy–naive 24 weeks after diagnosis with mortality in age- and sex-matched control subjects in the general population. The model assumed that after 24 weeks, individuals would initiate antiretroviral therapy at CD4+ cell counts of less than 350/μL.

Baseline characteristics were as follows: 80.4% men; country of birth was Western in 64.3%, sub-Saharan African in 15.5%, and other in 20.2%. The median CD4+ cell count at baseline was 480/μL, and 90.4% were asymptomatic. The final analysis included 4174 patients without an AIDS-defining condition 24 weeks after HIV infection diagnosis, and without a history of injection drug use (IDU). There were 17,580 person-years of follow-up. The mortality rate was 6.7 per 100 person-years. There were 118 deaths in the HIV-infected patients and 35 deaths in the matched control subjects. Prognostic variables associated with death included older age (HR, 1.07/year; 95% CI, 1.05 – 1.10), nonwestern country of birth (HR, 4.9; 95% CI, 2.3 – 10.4), and Centers for Disease Control and Prevention (CDC) stage B disease at 24 weeks (HR, 4.9; 95% CI, 2.1 – 11.5).

The expected median number of remaining life-years from age 25 years was 53.1 years in the general population and 52.7 years in the HIV-infected cohort. For those receiving a diagnosis at age 25 years, HIV infection was associated with 0.4 year of life lost; when diagnosed at age 55 years, HIV infection was associated with 1.3 years of life lost compared with the general population. More years of life were lost in HIV-infected women than men. The authors concluded that asymptomatic, HIV-infected patients who remain antiretroviral therapy–naive and have CD4+ cell counts above 350/μL at 6 months after diagnosis have life expectancies similar to those of the general population.

Lewden and colleagues performed a similar analysis comparing rates of death in HIV-1–infected patients receiving antiretroviral therapy with mortality rates in the general population (Abstract 527). Utilizing data from the COHERE (Collaboration of Observational HIV Epidemiological Research Europe) network of 25 European observational cohorts, the authors compared age- and sex-specific death rates among HIV-1–infected adults who received their first antiretroviral regimen after 1998. More than 80,000 patients in the cohort (85%) were eligible for analysis.

Baseline characteristics were as follows: 30% were women; median age was 37 years; 16% were IDU; and median CD4+ cell count was 225/μL. The median duration of follow-up was 3 years. Standardized mortality ratios (observed to expected mortality) were calculated according to CD4+ cell count strata and time spent with a CD4+ cell count of 500/μL or more. The standardized mortality ratios (SMRs) were higher in HIV-1–infected patients at all CD4+ cell count strata. However, the largest differences were observed in subjects with CD4+ cell counts below 200/μL and in women. Men with CD4+ cell counts of 500/μL or more at all time points had an SMR of 1.1 (95% CI, 0.8 – 1.3). IDUs of both sexes had substantially higher SMRs than those of non-IDUs at all CD4+ cell count strata. Although death rates in the COHERE patients did not differ by sex, SMRs were higher for women. The authors postulate that this difference may stem from socioeconomic status or other unmeasured confound-

ers. Even after 5 years at a CD4+ cell count stratum above 500/μL, HIV-infected women had an SMR of close to 2; in other words, a mortality rate twice as high as that for HIV-uninfected women in the general population. The authors concluded that HIV-infected men, but not women, achieve mortality rates similar to those of the general population.

Miro and colleagues presented data on clinical progression among persons receiving concomitant diagnoses of HIV and AIDS (Abstract 529). In this retrospective multicohort study of patients from Italy, Spain, the United Kingdom, and Canada, the authors examined the effect of timing of antiretroviral therapy initiation on prognosis in patients presenting with an AIDS-defining illness (ADI) at the time of HIV diagnosis. The primary outcome was clinical progression, defined as a new ADI or death.

The analysis included patients who received a diagnosis of HIV infection between 1997 and 2004 and had an ADI within 30 days before or 14 days after the HIV diagnosis. Immediate antiretroviral therapy was defined as being administered within 30 days of an ADI, whereas deferred antiretroviral therapy was defined as beginning between 31 days and 270 days after an ADI diagnosis. Deaths between days 0 and 30 were excluded from the analysis, but deaths occurring between days 31 and 270 were included. There were 429 subjects who met the criteria for inclusion: 77% men; 10% IDU; median age at diagnosis, 39 years; and median CD4+ cell count, 36/μL. ADIs included tuberculosis (TB) in 21.7%, *Pneumocystis jiroveci* pneumonia in 40.6%, Kaposi sarcoma in 13.9%, and lymphoma in 2.3%. PI-based therapy was used in 62.9% and NNRTI-based therapy in 28.2%. The median follow-up period was 2 years. Older patients and those with Kaposi sarcoma were more likely to start antiretroviral therapy earlier than other groups.

In the multivariate analysis, deferred treatment was associated with clinical progression (HR, 1.85; $P < .002$). Other factors associated with clinical progression in the multivariate analysis were as follows: lymphoma as the ADI (HR, 2.51; $P < .05$), age (HR per 5 years older, 1.10; $P = .02$), and viral load (HR per 1 $\log_{10}$ higher, 1.30; $P < .006$). The authors did not report the number of deaths, and they acknowledged that excluding deaths in the first 30 days of treatment renders the results applicable only to those surviving beyond 30 days. These data serve as observational support to the results of ACTG A5164 and suggest that antiretroviral therapy initiation proximal to the event of an ADI is associated with reduced mortality and new clinical events.

Palella and colleagues examined the relationship between baseline CD4+ cell count at antiretroviral therapy initiation and subsequent CD4+ cell count response and mortality (Abstract 983). Data from 1378 patients enrolled in the HOPS (HIV Outpatient Study) cohort who received antiretroviral therapy between 1996 and 2007 and had more than 6 months of follow-up while on antiretroviral therapy were included. The cohort consisted of 78.3% men; 33.6% black, 49.7% white, and 12.5% Hispanic. HIV transmission risk factors included 30.1% heterosexual, 56.2% men who have sex with men (MSM), and 6.5% IDU. Sixty percent carried private health insurance.

In the multivariate analysis, lower CD4+ cell count at the start of treatment was associated with a higher risk of death: for CD4+ cell counts below 50/μL at baseline, the HR was 4.6 (95% CI, 2.68 – 7.90) and for CD4+ cell counts between 50/μL and 199/μL, the HR was 2.6 (95% CI, 1.47 – 4.81) compared with those starting antiretroviral therapy at CD4+ cell counts above 200/μL. Having public health insurance was associated with a HR of death of 1.73 (95% CI, 1.11 – 2.70).

In the CD4+ cell count strata above 200/μL at treatment initiation, 76% of deaths were non–AIDS related. The median CD4+ cell count near death was 27/μL for persons with an AIDS-related death and 193/μL for those with non–AIDS related causes ($P < .001$). At 5 years of follow-up, the median CD4+ cell count of those starting with a pretreatment CD4+ cell count below 50/μL was 350/μL, and the median CD4+ cell count of those initiating therapy at a CD4+ cell count between 350/μL and 500/μL was 650/μL. At 5 years, 70% of patients whose CD4+ cell count was below 50/μL at baseline maintained CD4+ cell counts below 500/μL, compared with 20% of those starting antiretroviral therapy at CD4+ cell counts between 200/μL and 350/μL. The authors concluded that a lower CD4+ cell count at antiretroviral therapy initiation was associated with a lower long-term CD4+ cell count rise and increased mortality.

Many poster abstracts presented data on the relationship between low-level viremia and immunologic and clinical outcomes. In addition, several studies attempted to look at viral dynamics in patients with plasma HIV-1 RNA levels below the limit of detection who were receiving effective antiretroviral therapy.

Zhang and colleagues presented data on the impact of viremic episodes on CD4+ cell counts and non–AIDS-defining events in patients receiving suppressive antiretroviral therapy (Abstract 503). The subjects were 6440 patients selected from the ATHENA cohort. Inclusion criteria were as follows: taking initial therapy, plasma HIV-1 RNA level below 50 copies/mL by 48 weeks of antiretroviral therapy, and no history of non–AIDS-defining event. Subjects were censored at 1 year of treatment interruption. The authors defined 4 levels of viral outcomes: suppressed, low-level viremia (50 – 400 copies/mL), high-level viremia (> 400 copies/mL), and treatment interruption. Baseline characteristics were as follows: 75.3% men; median age, 39 years; median CD4+ cell count at start of treatment, 200/μL, and at viral suppression, 330/μL. Eighty-five percent of follow-up time was spent in virologic suppression. The authors noted higher rates of cardiovascular disease, but not renal or liver disease, in the high-level viremia group than in the suppressed group. These associations were independent of CD4+ stratum.

A large Canadian cohort showed higher mortality in patients with persistent low-level viremia (plasma HIV-1 RNA levels, 200 – 400 copies/mL

in 25% – 75% of measurements) than in patients with transient low-level viremia (< 25% of measurements with aforementioned parameters) (Abstract 504). However, mortality data for other viral load strata were not reported. Another study looked at subsequent virologic failure in patients with plasma HIV-1 RNA levels below 50 copies/mL (Abstract 505). The authors observed that an HIV RNA level between 40 copies/mL and 49 copies/mL was associated with a greater risk of subsequent virologic failure than were levels below 40 copies/mL.

Mavigner and colleagues compared residual viremia in 23 virologically suppressed patients with good versus poor immunologic responses (Abstract 307). The subjects had plasma HIV-1 RNA levels below 50 copies/mL while receiving antiretroviral therapy. Good immunologic response was defined as increases in CD4+ cell counts by more than 500/µL with treatment, whereas poor immunologic response was defined as gains of less than 200/µL. Quantification of residual viremia was performed with an ultrasensitive real-time polymerase chain reaction (RT-PCR) assay with a detectability range of 2.5 copies/mL. A nested RT-PCR was used to amplify the V3-loop of *env* to determine coreceptor use. Low-level viremia was detected in 13 of 23 patients, with higher levels (but not higher frequency) of residual HIV-1 RNA detected in poor than in good responders ($P < .05$). Residual viremia was associated with CD4+ cell activation in poor responders only. The authors also observed viral evolution to X4-tropic virus in these virologically suppressed subjects. No data on the clinical history of the patients were presented.

Antiretroviral Treatment in Resource-Limited Settings

Treatment Scale-up in Resource-Limited Settings

Antiretroviral therapy scale-up in RLS continued to be a focus at this year's conference. A number of plenary addresses and oral presentations emphasized the achievements and the gaps in antiretroviral therapy coverage in RLS. Fauci reported that approximately 4 million people in low- and middle-income countries were receiving antiretroviral therapy in 2008 (Abstract 19). This figure represents an exponential rise in antiretroviral therapy availability in RLS, from less than 1 million people receiving antiretroviral therapy as recently as 2002. However, the 4 million people now receiving antiretroviral therapy in RLS represent only 40% of those meeting guidelines for treatment initiation (ie, at a CD4+ cell count threshold of less than 200/µL). Fauci emphasized that with evolving guidelines recommending a treatment initiation threshold of less than 350/µL, only 30% of those meeting criteria for treatment in RLS are currently receiving antiretroviral therapy.

Delay of UNAIDS (The Joint United Nations Programme on HIV/AIDS) projected that 15 million people would meet criteria for antiretroviral therapy initiation in RLS under new treatment guidelines (ie, at a CD4+ cell count of 350/µL or below), accounting for nearly half of people living with HIV (Abstract 18). In a session dedicated to a discussion of the future of PEPFAR (President's Emergency Plan for AIDS Relief), Delay noted that $16 billion (US) has been committed to provide antiretroviral therapy in low- and middle-income countries (Session 4). In a session focusing on the global response to the HIV pandemic, Sow reviewed challenges and limitations of scale-up efforts, including low CD4+ cell counts at treatment initiation, use of initial regimens that have high levels of adverse effects, presence of coinfections, absence of plasma HIV-1 RNA monitoring, and limited options for second-line treatment (SLT) (Abstract 15).

Several qualitative and quantitative evaluations of antiretroviral therapy scale-up programs were presented. In Session 28, Ingle and colleagues presented data on mortality among patients awaiting antiretroviral therapy initiation after enrollment in a provincial treatment program in South Africa (Abstract 108). The study aims were to assess the cumulative proportion of patients starting antiretroviral therapy, as well as the cumulative mortality of those awaiting treatment initiation. The setting was an antiretroviral therapy program in the Free State Province of South Africa, where the antiretroviral therapy initiation eligibility threshold is a CD4+ cell count below 200/µL.

Approximately 22,000 patients were enrolled between 2004 and 2007 and observed until 2008. Sixty-four percent of subjects were women. The disposition of patients after 2 years of follow-up was reported as follows: 26% died before antiretroviral therapy initiation, 68% started treatment, and only 6% were alive and untreated. Risk of death was much higher at lower CD4+ cell count strata. For example, among those with pretreatment CD4+ cell counts of less than 25/µL, 46% of patients had died before initiating antiretroviral therapy.

In a model adjusted for baseline characteristics, factors associated with a lower likelihood of initiating antiretroviral therapy included male sex and a CD4+ cell count below 25/µL. Higher rates of pretreatment deaths were seen at lower CD4+ cell count strata, among men, and in older patients, even after adjusting for likelihood of antiretroviral therapy initiation. The median CD4+ cell count at eligibility to initiate therapy was 101/µL, because of a median time of 183 days between CD4+ cell count measurements. Causes of death were not described in the presentation. The presentation highlighted the substantial loss of life in those awaiting antiretroviral therapy initiation; the authors suggested that guidelines raising the CD4+ cell count treatment threshold would lead to earlier access and may reduce pretreatment mortality.

Serenata and colleagues evaluated progress toward meeting the September 2009 PEPFAR-South Africa target of providing antiretroviral therapy to 456,571 patients (Abstract 828). The authors also looked at rates of antiretroviral therapy continuation within the program. In June 2005, 120,904 patients were receiving antiretroviral therapy under the program. The authors report that by September 2009, 646,972 patients were receiving treatment, representing a 435% increase in

coverage, exceeding the set target by 42%. Sixty-seven percent of patients were women. Of those who initiated treatment, 4.4% died, 8.0% were LTFU, 1.4% stopped treatment, and 1.1% had an unknown status. The overall rate of antiretroviral therapy continuation was 77%. Government-run treatment sites showed a slightly higher rate of continuation than did private clinics or those managed by nongovernment organizations. Overall, rates of coverage and continuation were excellent and exceeded set targets.

Primary Treatment Outcomes in Resource-Limited Settings

Five-year follow-up data from the DART (Development of Antiretroviral Therapy) trial in Africa trial were presented by Munderi and colleagues (Abstract 110). DART is a randomized trial of 2 strategic approaches to antiretroviral therapy management—a clinically driven approach and a laboratory-enhanced monitoring strategy. The data presented in this session pertained to treatment outcomes in 3316 adults enrolled in the study in Uganda and Zimbabwe. Patients were included if they were antiretroviral therapy–naive with advanced immune deficiency by clinical staging and had a CD4+ cell count below 200/μL. Median pretreatment CD4+ cell count was 86/μL, with one-third of subjects having a CD4+ cell count below 50/μL. All subjects received zidovudine/lamivudine with the following distribution of the third drug: tenofovir in 74%, nevirapine in 16%, and abacavir in 9%.

The authors presented data on immune restoration and the estimated time to attainment of prespecified CD4+ cell count stratum change in subjects remaining on the initial regimen. The median follow-up time was 4.7 years. At follow-up, 301 patients had died, 108 were LTFU, and 697 switched to a second regimen. Of those patients remaining on initial therapy at 5 years, 69% ever achieved a CD4+ cell count above 250/μL, 46% ever achieved a CD4+ cell count above 350/μL, and only 19% ever achieved a CD4+ cell count above 500/μL. The authors note that, even in subjects with the highest baseline CD4+ cell counts (150 – 200/μL), less than 75% achieved CD4+ cell counts above 350/μL.

Using receiver operating characteristic analysis, the authors sought to determine a CD4+ cell count threshold at 1 year of treatment that would predict failure to attain a CD4+ cell count above 250/μL at 5 years. This analysis identified a CD4+ cell count cut-off of less than 125/μL as having the best operating characteristics for predicting failure to attain the prespecified threshold. The operating characteristics were as follows: specificity, 93%; sensitivity, 42%; positive predictive value, 74%; and negative predictive value, 78%. The rate of CD4+ cell count increase did not identify those who would attain a CD4+ cell count above 250/μL. Once again, these data suggest that treatment at higher CD4+ cell count thresholds would be associated with more robust restoration of immunologic parameters.

Renaud-Thery and colleagues presented a systematic review of the literature on antiretroviral therapy outcomes in cohort studies in RLS (Abstract 827). Inclusion criteria were publications reporting on antiretroviral therapy–naive subjects prescribed WHO-defined initial regimens. Outcomes of interest included treatment failure and attrition rates. Failure was defined using WHO criteria for clinical, immunologic, and virologic failure (for definitions, see Evaluation of World Health Organization Clinical and Immunologic Criteria for Treatment Failure of Antiretroviral Therapy below). Attrition was defined as death, LTFU, transfer, or discontinuation. Retention was defined as alive and taking any antiretroviral therapy. A total of 804 citations were identified, representing 124,491 patients. Five studies were cross-sectional, with the remainder being observational cohorts. Sixty-six percent of studies reported data from Africa. Overall the clinical and immunologic failure rate was 1.9 per 100 person-years, and the virologic failure rate was 6.08 per 100 person-years. Attrition rate was 19.6 per 100 person-years. Failure and attrition rates were higher in Africa than in Latin America and Asia. The authors urged cautious interpretation of the results because of the heterogeneity of the studies included.

Cortes and colleagues presented national data on 3045 patients receiving initial antiretroviral therapy since 2001 in Chile's expanded access antiretroviral therapy program (Abstract 523). In this cohort, 84.7% of patients were men, and the median age was 36.9 at enrollment. Outcome measures included mortality, LTFU, switch of initial regimen, viral suppression, and immune recovery over the first 4 years of treatment. At 48 months, 84.2% remained enrolled in the program. Of those, 86% achieved a plasma HIV-1 RNA level below 400 copies/mL, and 81% achieved a level below 80 copies/mL. Eleven percent had died, and 4.3% were LTFU. NNRTI-based regimens were used by 64% of patients, and indinavir-based regimens by 15%. At 48 months, 60% of patients remained with their initial regimen. The most common cause for regimen switch was an adverse event. At 48 months, the median CD4+ cell count was 350/μL.

Chinh and colleagues looked at mortality and treatment durability in 889 patients starting an initial antiretroviral regimen at a hospital-based site in Vietnam (Abstract 517). Seventy-seven percent of patients were men, 80% were IDUs, and 51% had WHO stage 3 or 4 disease at baseline. The baseline median CD4+ cell count was 122/μL, and 77% had prior antiretroviral therapy exposure (although the authors do not characterize past regimens). Median duration of treatment was 10 months, with all patients receiving NNRTI-based therapy.

The following outcomes were observed: 5% of patients died, 4% were LTFU, 2.1% had treatment failures and 1.2% transferred care. At 1 year, 88% of participants were still in follow-up and on initial antiretroviral therapy. Not surprisingly, advanced disease at baseline was predictive of subsequent death. No difference in the probability of regimen switch was observed between the antiretroviral–naive and –experienced groups. In this mostly pretreated cohort with a majority re-

porting IDU, high rates of retention in care and low rates of treatment failure were reported.

Outcome Predictors in Resource-Limited Settings

Many poster presentations examined predictors of treatment outcomes in RLS. Fox and colleagues presented data on outcomes in patients initiating antiretroviral treatment at different CD4+ cell count strata in a prospective cohort of 812 patients in the CIPRA-SA (Comprehensive International Programme for Research on AIDS – South Africa) trial (Abstract 521). This randomized, controlled trial compared physician-monitored with nurse-monitored HIV care in South Africa. The trial enrolled antiretroviral treatment–naive adults with a CD4+ cell count below 350/μL or a previous ADI. Ninety-two percent of subjects received NNRTI-based therapy for a maximum follow-up period of 252 weeks. No differences were found between the physician- and nurse-monitored groups.[5]

The authors now presented data on treatment outcomes in 2 nonrandomized groups of patients: those who started treatment at CD4+ cell counts below 200/μL, and those who started treatment at CD4+ cell counts between 200/μL and 350/μL. Outcome measures included treatment failure (defined as death or virologic failure), incident TB, and program failure (defined as patients with more than 3 missed visits). At baseline, and by definition, patients in the low CD4+ cell count group had more advanced disease, but no other differences were noted in age, sex, or treating practitioner. Adjusted HR for death or virologic failure was 2.13 (95% CI, 1.3 – 3.6) in the CD4+ cell count group below 200/μL compared with those in the group above 200/μL. The HR for death was 5.39 (95% CI, 1.26 – 22) between the low- and high-CD4+ cell-count groups (the authors did not report on adjustments made). The adjusted HR for incident TB was 2.59 (95% CI, 1.28 – 5.26) in the lower compared with the higher CD4+ cell count strata (although data presented in the Kaplan-Meier analysis showed less follow-up time in the higher CD4+ cell-count stratum). In total, these data provide observational support to current WHO guidelines for initiation of antiretroviral therapy.

Takuva and colleagues explored the relationship between initial CD4+ cell count response and subsequent survival in adults receiving initial antiretroviral therapy in a public sector clinic in South Africa (Abstract 520). Patients treated with an initial regimen between 2004 and 2009 and who achieved a plasma HIV-1 RNA level below 400 copies/mL by 6 months of treatment were included in the analysis. The authors defined 2 response groups: those with and those without an increase in CD4+ cell count of more than 50/μL at 6 months of treatment. Outcome measures included death, ADI, and a composite outcome of death and ADI. More than 6000 patients were included in the analysis. An increased risk of death, ADI, and composite death and ADI was observed in the group with a CD4+ cell count gain of less than 50/μL at 6 months, despite virologic suppression. The HR for death was 1.95 (95% CI, 1.25 – 3.04) between the group without and the group with gains of at least 50/μL. The analysis controlled for age, sex, baseline CD4+ cell count, hemoglobin (hgb) level, and TB treatment status.

May and colleagues created a prognostic model for predicting mortality during the first year of antiretroviral therapy in sub-Saharan Africa (Abstract 815). Using data from more than 10,000 patients enrolled in antiretroviral therapy scale-up programs in Cote d'Ivoire, Malawi, and South Africa, the authors created 2 models predictive of mortality: a CD4+ cell-count inclusive model, and a model based on hgb level and total lymphocyte count (TLC). Both models also incorporated the variables of age, sex, WHO stage 3 or 4 disease at baseline, and body weight. The baseline characteristics of the cohort were 68% women; median age, 34 years; 85% with advanced disease, and median pretreatment CD4+ cell count, 111/μL. During the first year of follow-up, 8.2% of patients died. Observed and predicted mortality tracked closely with one another. Both models predicted mortality accurately, and the hgb-TLC model predicted mortality as accurately as did the CD4+ cell-count–based model. The authors plan to make the model available as a Web-based calculator at www.iedea-sa.org.

Another approach to outcome prediction was presented by Koethe and colleagues, who looked at pretreatment body mass index (BMI) and subsequent mortality and CD4+ cell count gains (Abstract 819). More than 33,000 adults in Zambia who initiated antiretroviral therapy between 2004 and 2009 were included in the analysis if they had received antiretroviral therapy for at least 6 months and had a CD4+ cell count measurement at 6 months of treatment. The cohort was stratified according to WHO categories for malnutrition based on pretreatment BMI.

Sixty-two percent of patients were women, median age was 35 years, and median BMI was 20.1 kg/m^2. At baseline, median CD4+ cell count was 142/μL, 13% of patients had active TB, and 37.2% had WHO stage 1 or 2 disease. No statistically significant difference was found in CD4+ cell count gains at 6 months among the different BMI strata. However, the risk of death was statistically significantly higher in malnourished individuals unable to achieve a gain in CD4+ cell count of at least 100/μL. The highest risk of death by BMI stratum was in subjects with a baseline BMI below 16 kg/m^2 (severely malnourished) and a decline in CD4+ cell count at 6 months, with an HR of death of 6.08 (95% CI, 3.59 – 10.3). All analyses were adjusted for age, sex, baseline hgb level, WHO clinical stage, TB infection, initial antiretroviral therapy regimen, and adherence. The synergistic relationship between nutritional status (BMI), low CD4+ cell count gain, and mortality underscores the importance of integrating nutritional support and food security into health systems providing antiretroviral therapy.

Two studies found a relationship between clinic attendance and mortality. Brennan and colleagues conducted a retrospective analysis of 7788 adults initiating antiretroviral therapy in South Africa (Abstract 821). All pa-

tients were antiretroviral therapy–naive at baseline and attended clinic for at least 6 months after antiretroviral therapy initiation. A 2-fold increase in mortality was observed in patients missing more than 1 visit in the first 6 months of treatment. A similar but much larger study in a RRS looked at the relationship between early missed visits and mortality in China (Abstract 822). Twenty-seven thousand antiretroviral treatment–naive adults initiating antiretroviral therapy were included: 61% were men, median age was 38.6 years, and baseline CD4+ cell count was 126/μL. After adjustments for numerous variables, any missed visit in the first 6 months was associated with an increase in mortality over 60 months of observation. Missing 3 or more visits was associated with a HR of death of 1.72 (95% CI, 1.36 – 2.16).

Two posters compared treatment outcomes in RLS and RRS. Geng and colleagues compared the immunologic efficacy of antiretroviral therapy in a North American setting with that of an African treatment setting (Abstract 518). A total of 8457 patients contributed a median of 2.2 years of observation. Patients included in the analysis had a baseline CD4+ cell count below 350/μL and had achieved a plasma HIV-1 RNA level below 1000 copies/mL within 1 year of treatment. Baseline characteristics differed between African and North American sites in sex (68% and 19% women, respectively) and baseline CD4+ cell count (122/μL and 161/μL, respectively). At 36 months, African patients showed slight but statistically significantly greater gains in CD4+ cell counts than those of their North American counterparts. The authors commented, however, that an opposite trend was seen in the first year of treatment and postulate that comorbid infections in the African setting may have contributed to the slower gains. There was no mention of the impact of baseline characteristics on the findings.

Wester and colleagues compared rates of non–AIDS-defining events in patients receiving antiretroviral therapy in Botswana and the United States (Abstract 726). Baseline characteristics differed between the Botswana and US groups with respect to sex (69% and 26% women, respectively), ethnicity (100% and 36% black, respectively), BMI (21.3 and 24.5 kg/m^2, respectively), and median CD4+ cell count (199/μL and 243/μL, respectively). Non–AIDS-defining events were adjudicated by an endpoints committee in Botswana and by *International Classification of Diseases, Ninth Revision (ICD-9)* code extraction in the United States. Crude incidence of non–AIDS-defining events was 9.99 per 100 person-years in Botswana and 12.2 per 100 person-years in the United States. Higher rates of hepatic and renal disease were observed in the United States whereas higher rates of malignancies were observed in Botswana. There was higher mortality in African patients.

A number of abstracts looked at HIV treatment outcomes in patients coinfected with the viral hepatidites. Chadwick and colleagues examined HIV treatment outcomes in HIV monoinfected and HIV-HBV coinfected patients receiving antiretroviral therapy in Ghana (Abstract 690). Prevalence of HBV coinfection at the treatment site was 17%. No differences were observed in clinical, immunologic, or virologic HIV treatment outcomes between the mono- and coinfected groups. Because most patients were receiving de facto HBV monotherapy with lamivudine as part of their antiretroviral regimen, the authors performed HBV genotypic analysis in a subset of coinfected patients. They found 18% of their HBV sample to have lamivudine RAMs.

On the other hand, Christian and colleagues showed slower rates of immune recovery in nearly 5000 HIV-infected patients coinfected with either HBV or HCV and receiving antiretroviral therapy through PEPFAR-supported sites in Tanzania (Abstract 694). Nguyen and colleagues conducted a retrospective review comparing mortality in HIV monoinfected and HIV-HCV coinfected patients receiving antiretroviral therapy in Vietnam (Abstract 817). Almost 2000 patients treated between 2005 and 2008 were included in the analysis. Twenty-seven percent were coinfected. Coinfected individuals were more likely than HIV-monoinfected patients to be men, have a history of IDU (76% and 33%, respectively), and have higher baseline CD4+ cell counts (79/μL and 71/μL, respectively) and alanine aminotransferase levels. At a median follow-up of 15.7 months, 14% of the sample had died, and 5.2% were LTFU. LTFU was more frequent in coinfected patients, even after controlling for IDU status. There were no differences in mortality between the groups. CD4+ cell counts at follow-up did not differ between the groups, but monoinfected individuals had larger CD4+ cell count gains. The duration of follow-up in this study was likely too short to observe differences in mortality.

Outcomes of Second-Line Therapy in Resource-Limited Settings

There was a paucity of data on second-line treatment outcomes in RLS. Pujades-Rodriguez and colleagues presented mortality and virologic outcome data in patients receiving SLT at 27 Medecins sans Frontieres–supported sites (Abstract 524). Adults (n = 632) who were antiretroviral therapy–naive at treatment initiation, received NNRTI-based initial treatment, were subsequently switched to PI-containing SLT, and had at least 6 months of follow-up while receiving SLT were included in the analysis. All programs had routine CD4+ cell-count monitoring, and 4 sites tested for plasma HIV-1 RNA level. SLT failure was defined using 2006 WHO criteria for clinical, immunologic, and virologic failure. The primary outcome was SLT failure and mortality after 6 months of treatment. Median follow-up time with SLT was 35 months.

Nineteen percent of subjects met any criteria for treatment failure, 5% had died, and 4% were LTFU. Factors associated with SLT failure included lower CD4+ cell count at start of SLT; factors inversely associated with failure included hospital-based setting (compared with health care center), changing more than 1 nRTI at time of switch to SLT (compared with a switch of 1 nRTI), and use of lopinavir/r (com-

Table **2.** Selected Studies on Antiretroviral Treatment Outcomes in Resource-Limited Settings

Abstract No. Study Title	Research Question or Aim	Study Design (no. participants) Participating Locations	Findings
Abstract 153LB. Efficacy of ART with NVP + TDF/FTC vs LPV/r + TDF/FTC Among Antiretroviral-Naive Women in Africa: OCTANE Trial 2/ACTG A5208	Is there equivalence in efficacy between nevirapine + tenofovir/emtricitabine and lopinavir/r + tenofovir/emtricitabine in ART-naive women without exposure to sdNVP as part of PMTCT?	Randomized, controlled, open-label equivalence study (n = 500) South Africa	For primary endpoint of death or virologic failure, HR was 0.82 comparing nevirapine to lopinavir/r both in combination with tenofovir/emtricitabine; this finding met prespecified criteria for equivalence
Abstract 110. Immune Restoration Over 5 Years on ART Among Patients Initiating Treatment With Advanced Immune Deficiency in the DART Trial in Uganda and Zimbabwe	What are the CD4+ cell count attainments in persons with a pretreatment CD4+ count < 200/µL who initiate ART in Africa?	Parent study (DART) is a randomized, controlled trial comparing clinical versus laboratory-driven monitoring of HIV-infected patients initiating ART. Current analysis presents observational data on CD4+ cell count stratum attainment (n = 3316) Uganda, Zimbabwe	At median follow-up of 4.7 years, 66% of subjects remained on initial regimen 69% ever achieved a CD4+ cell count > 250/µL 19% ever achieved a CD4+ cell count > 500/µL A pretreatment CD4+ cell count threshold < 125/µL was most predictive of failure to achieve CD4+ cell count > 250/µL
Abstract 827. Adult ART in Resource-Limited Settings: A Systematic Review of First-Line Treatment Failure and Attrition Rates	To perform a systematic review of the literature on initial ART outcomes in resource-limited settings	Meta-analysis of published data on outcomes in ART-naive subjects receiving initial therapy in resource-limited settings (n = 804 citations reflecting 124,491 subjects) Africa, Latin America, Asia	Primary outcome was treatment failure as defined by WHO criteria Clinical and immunologic failure rate: 1.9/100 person-years Virologic failure rate: 6.08/100 person-years Attrition rate (including death, loss to follow-up, transfer, or discontinuation): 19.6/100 person-years
Abstract 521. Effect of Initiating Patients on HAART at CD4 Counts Above 200 on Virologic Failure and Death in South Africa: Evidence from the CIPRA-SA Trial	Are there differences in treatment outcomes in ART-naive adults initiating therapy at CD4+ cell counts < 200/µL vs < 350/µL?	Parent study (CIPRA-SA) was a randomized, controlled trial comparing nurse- versus physician-monitored care; current analysis compared 2 nonrandomized groups of patients: those who initiated ART at a CD4+ cell count < 200/µL and those who initiated at a CD4+ cell count between 200/µL and 350/µL (n = 812) South Africa	Primary outcome measures included treatment failure, incident TB, and loss to follow-up Comparing the CD4+ cell count < 200/µL group with the < 350/µL group, HRs were: Death or virologic failure, adjusted HR = 2.13 Death, unadjusted HR = 5.39 Incident TB, adjusted HR = 2.59
Abstract 524. Failure to Second-Line Therapy and Associated Mortality in 27 MSF-Supported African and Asian ART Programs	To determine treatment outcomes in HIV-infected patients receiving second-line ART in resource-limited settings	Retrospective analysis of patients receiving second-line ART in Médecins sans Frontières–supported clinical sites (n = 632) Africa, Asia	Primary outcome was treatment failure as defined by WHO criteria. At median follow-up of 35 months, 19% met any criteria for failure, including 5% who had died Treatment failure was associated with lower CD4+ cell count, use of nelfinavir (compared with lopinavir/r), and switch of 1 nRTI (compared with switch of 2 nRTIs) at time of switch to second-line therapy

ACTG indicates AIDS Clinical Trials Group; ART, antiretroviral therapy; CIPRA-SA, Comprehensive International Programme for Research on AIDS–South Africa; DART, Development of Antiretroviral Therapy; FTC, emtricitabine; HAART, highly active antiretroviral therapy; HR, hazard ratio; LPV, lopinavir; MSF, Médecins sans Frontières; nRTI, nucleoside analogue reverse transcriptase inhibitor; NVP, nevirapine; OCTANE, Optimal Combination Therapy After Nevirapine Exposure; PMTCT, prevention of mother-to-child transmission; /r, ritonavir-boosted; sdNVP, single-dose nevirapine; TB, tuberculosis; TDF, tenofovir; WHO, World Health Organization

pared with nelfinavir). Mortality was strongly associated with immunovirologic failure, but all types of WHO failure criteria were associated with an increased risk of death. The authors commented that rates of failure of SLT were 46% higher than those of initial antiretroviral therapy, but did not present these data. This study and others presented on treatment outcomes in RLS are summarized in Table 2.

Evaluation of WHO Clinical and Immunologic Criteria for Treatment Failure of Antiretroviral Therapy

The 2006 WHO definition of treatment failure includes clinical, immunologic, and virologic criteria. Clinical treatment failure is defined as new stage 3 or 4 events after 6 months of treatment. Immunologic failure is defined as any of the following: CD4+ cell count decrease to pretherapy baseline level, a CD4+ cell count consistently below 100/μL, or a 50% fall from pretreatment peak after 1 year of treatment. Virologic failure is defined as a plasma HIV RNA level above 10,000 copies/mL after 6 months of treatment.

A number of presentations evaluated the accuracy of the clinical and immunologic WHO failure criteria in predicting virologic failure. Rawizza and colleagues presented data assessing the performance of immunologic criteria in predicting virologic failure in the Harvard–PEPFAR treatment program (Abstract 111). All patients undergo CD4+ cell count and plasma HIV-1 RNA measurements at baseline, at 3 months and 6 months, and every 6 months thereafter. In this study, virologic failure was defined as 2 consecutive plasma HIV-1 RNA measurements above 1000 copies/mL after at least 6 months of antiretroviral therapy. The analysis included 9690 antiretroviral therapy–naive adults. The median follow-up time was 33 months. Virologic failure was observed in 25.9% and immunologic failure in 32.2%. WHO CD4+ cell count failure criteria had a sensitivity of 54.9% and specificity of 75.7%, a positive predictive value of 44.2%, and a negative predictive value of 82.7% for virologic failure. In other words, CD4+ cell count criteria missed 45.1% of virologic failures and misclassified 44.2% as failures that were in fact virologically suppressed. Furthermore, plasma HIV-1 RNA level monitoring identified failure earlier than immunologic criteria by a mean of 4.2 months ($P < .001$).

Further data on the poor predictive value of immunologic criteria for predicting virologic failure were presented by Eisenberg in a cross-sectional study from a Medecins sans Frontieres–supported treatment site in Kenya (Ferreyra et al, Abstract 816). Included in the analysis were 926 antiretroviral therapy–naive adults who were initiated on treatment with NNRTI-based therapy and had a follow-up period of at least 12 months. The cohort consisted of 67.3% men, with a median age at treatment initiation of 38 years and baseline CD4+ cell count of 133/μL. At 12 months, 83.9% of participants had a plasma HIV-1 RNA level below 400 copies/mL. At a median follow-up time of 38 months, 13.3% of patients experienced clinical failure, 5.7% immunologic failure, and 4.7% virologic failure. The sensitivity of immunologic and clinical criteria for diagnosing virologic failure was 23.6% and 18.2%, respectively; the specificity was 95.4% and 87%, respectively; and the positive predictive value was 24.5% and 8.1%, respectively. The use of the 2006 WHO definition of virologic failure (plasma HIV-1 RNA level > 10,000 copies/mL) would have led to an underestimation of more strictly defined criteria for virologic failure and may have falsely lowered the positive predictive value.

Laboratory Monitoring in Resource-Limited Settings

Chaiwarith and colleagues compared antiretroviral therapy outcomes in Thai patients undergoing once–versus twice–yearly monitoring of plasma HIV-1 RNA level as part of routine care (Abstract 500). The retrospective cohort analysis included 578 patients receiving antiretroviral therapy at a single hospital center in Thailand. Outcome measures included the incidence of virologic failure, time to virologic failure, and reverse transcriptase resistance mutations at failure. Virologic failure was defined as a plasma HIV-1 RNA level above 1000 copies/mL. In this cohort, 46.2% were men, the median age at baseline was 40 years, and baseline CD4+ cell count was 60/μL. Ninety-seven percent were receiving NNRTI-based therapy. Once-yearly plasma HIV-1 RNA level monitoring was performed in 73.4%, whereas twice-yearly monitoring was performed in 26.6%. There were no baseline differences in the monitoring groups with respect to age, sex, or first antiretroviral regimen.

At 7 years of follow-up, the incidence of virologic failure was 4.32 per 100,000 person-days in the once-yearly HIV-1 RNA monitoring group and 3.08 per 100,000 person-days in the twice-yearly group, ($P = .43$). Mutation rates between the groups did not differ. Virologic failure was highly associated with adherence rates of less than 95% (actual adherence rates were not reported). The authors concluded that in settings with high adherence rates, frequency of plasma HIV-1 RNA level monitoring does not affect rates of virologic failure. The authors did not comment on the timing of HIV-1 RNA measurements in the course of treatment.

Walker and colleagues presented additional data on the impact of laboratory monitoring and treatment outcomes in the DART trial (Abstract 56) (see "Primary Treatment Outcomes in Resource-Limited Settings" above). Briefly, antiretroviral therapy–naive adults with advanced disease underwent randomization to 1 of 2 monitoring approaches: a laboratory and clinical monitoring (LCM) group, which included routine monitoring of biochemistry parameters and CD4+ cell count, and a clinically driven monitoring (CDM) group, for which CD4+ cell counts were not available in real time and biochemistry analysis for toxicity was performed only when clinically indicated.

There was no difference in mortality between the 2 monitoring approaches in the first 2 years of follow-up, but after 2 years, the LCM group showed lower rates of death.

The current analysis tried to explain the difference in mortality. After 2 years of treatment, subjects in the CDM group spent more time with CD4+ cell counts below 200/μL than did those in the LCM group (39% versus 33% of follow-up time, respectively). No difference in risk of death at a given CD4+ cell count was observed between groups. However, more WHO-defined stage 4 events were observed in the CDM subjects but only at *higher* CD4+ cell counts.

Why was this counterintuitive relationship observed? The authors argue that the different rates of WHO stage 4 events between the CDM and LCM groups at higher CD4+ cell counts was due to an underestimation of events in the LCM subjects. In other words, the authors hypothesize that knowledge of a high CD4+ count in the LCM subjects led to a CD4+-dependent underreporting bias of WHO stage 4 events. They argue that this bias may have important implications for the measurement of outcomes in open-label randomized and observation studies.

Resistance in Resource-Limited Settings

Dlamini and colleagues investigated the prevalence and type of RAMs in patients in South Africa receiving initial antiretroviral therapy and who achieved virologic suppression and had a subsequent viral load rebound (Abstract 589). The authors also looked at virologic and immunologic responses of patients after viral rebound. The data were obtained from a subgroup of the South African Phidisa II trial subjects, which compared 4 different antiretroviral drug regimens in treatment-naive individuals with advanced HIV disease. In the parent Phidisa II study, 1771 subjects were enrolled and underwent randomization to receive efavirenz or lopinavir/r and zidovudine plus didanosine or lamivudine plus stavudine.

Included in this resistance substudy are 73 patients who met virologic failure criteria (plasma HIV-1 RNA level > 1000 copies/mL on 2 consecutive visits) after achieving viral suppression at 6 months of treatment. Of the 73 patients, 68 had genotypes available. At the time of virologic failure, 30 patients were receiving efavirenz-based regimens, and 31 were receiving lopinavir/r-based regimens (the remainder were not receiving antiretroviral therapy). In patients taking an efavirenz-based regimen at treatment failure, 56.7% had any reverse transcriptase mutations, and 36.7% had both NNRTI and nRTI RAM. In the lopinavir/r group, 16.1% had any reverse transcriptase mutations, but no PI RAMs were observed. The most common RAMs observed were K103N and M184V. Thirty-three percent of patients had no RAMs at failure.

Saravanan and colleagues looked at resistance mutations among patients in India for whom SLT was failing (Abstract 592). The study was a cross-sectional analysis of 107 patients receiving PI/r-based SLT in a setting the authors describe as having limited virologic monitoring. All patients with a plasma HIV-1 RNA level above 1000 copies/mL underwent genotypic analysis. The group consisted of 75% men, the mean age was 35 years, and 97% reported heterosexual risk factors. The median CD4+ cell count at failure was 146/μL, and the mean duration of PI exposure was 13 months. Fifty-one percent were receiving atazanavir/r, 46% indinavir/r, and 3% lopinavir/r. The nRTI backbone was tenofovir/emtricitabine in 55%, didanosine plus lamivudine in 24%, and zidovudine or stavudine with lamivudine in 21%.

Of the 107 patients, 45 (42%) experienced virologic failure, and 100% of those had any RAM at failure. Seventy-three percent had PI resistance mutations, 91% had nRTI resistance mutations, 73% had NNRTI resistance mutations, and 53% showed triple-class resistance. The rates of failure and RAMs reported in this analysis were far higher than described in the literature, especially with ritonavir-boosted, PI-based regimens. No data were provided regarding the treatment history of the patients included in the analysis. At failure, most patients were sensitive by phenotype to darunavir/r, but less than 50% were sensitive to lopinavir/r, suggesting the possibility of unreported PI exposure before current SLT.

Rosen and colleagues presented a Markov model comparing the cost-effectiveness of 2 initial therapy switch strategies: a viral load–based strategy, and a strategy incorporating viral load and resistance testing (Abstract 823). The models utilized clinical and cost data derived from a large clinical cohort in South Africa. In the viral load–based model, all patients with confirmed virologic failure are switched to SLT. In the model incorporating resistance testing, patients with virologic failure undergo genotypic analysis, but only patients with RAMs at failure are switched to SLT. The model assumed low failure rates and a 16% rate of RAMs at initial treatment failure. Modeling predicted that at 5 years, 20% of subjects experienced virologic failure. At this rate, resistance testing was found to be cost-saving, with approximately $33 (US) saved per patient. Interestingly, there was no statistically significant difference in switch rates to SLT between the strategies, with 20% and 17% switching in the viral load–versus the resistance-based strategy, respectively. In the sensitivity analysis, decreasing the rates of resistance at failure increased cost-savings, presumably because of lower rates of switch to SLT. The authors conclude that incorporating resistance testing into treatment algorithms in Africa will be either cost-saving or cost-neutral.

Mother-to-Child Transmission of HIV Infection

Incident HIV Infection During Pregnancy

Kinuthia and colleagues determined factors associated with incident HIV-1 infections during pregnancy and the postpartum period among women who brought their infants for 6-week routine vaccinations at maternal-child health clinics in Nairobi and Kenya (Abstract 155). Women who had tested HIV-seronegative during the antenatal period were evaluated. These women completed a questionnaire and received HIV testing. Acceptability of HIV testing was high (95.3%). Fifty-three (2.6%) of 2035 women who ac-

cepted HIV testing were HIV-seropositive, with a relatively high HIV-infection incidence of 6.8 per 100 person-years. On multivariate analysis, HIV seroconversion was statistically significantly associated with employment and residence in a high-HIV-prevalence region. Other factors such as age, marital status, education level, and economic well-being were not associated with seroconversion.

Six-Week Extended Nevirapine Studies

Twelve-month follow-up data from the SWEN (Six-Week Extended Nevirapine) Studies were presented by Bedri and colleagues (Abstract 157). The SWEN trials consisted of 3 randomized controlled studies in Ethiopia, India, and Uganda comparing extended-dose nevirapine through 6 weeks of age with single-dose nevirapine to prevent HIV transmission from HIV-infected mothers to their infants through breastfeeding. Six-week and 6-month data from these trials have been presented previously.[6,7] A total of 1890 infants were included: 903 in the SWEN (extended-dose) group and 987 in the single-dose group. Three 12-month endpoints were analyzed: HIV transmission, cumulative mortality, and a combined outcome of HIV transmission or mortality. No statistically significant difference in HIV transmission at 12 months was observed between the 2 groups (8.9% in the extended-dose group vs 10.4% in the single-dose group). Infants in the extended-dose group had a 47% lower cumulative mortality at 12 months than did those in the single-dose group. When stratified by maternal CD4+ cell count, mortality and the combined outcome of HIV transmission or mortality were lower among infants whose mothers had a CD4+ cell count of at least 350/μL.

Nevirapine Resistance Study

Moorthy and colleagues examined the effect of nevirapine resistance frequencies on virologic outcomes among HIV-infected children enrolled in the NEVEREST (Nevirapine Resistance Study) trial (Abstract 159). The NEVEREST trial involved HIV-infected children who had been exposed to single-dose nevirapine and received lopinavir/r–based antiretroviral therapy as their initial regimen. Children with virologic suppression for at least 3 months underwent randomization to starting nevirapine-based antiretroviral therapy or continuing lopinavir/r–based antiretroviral therapy. In this analysis, the authors focused on children who were switched to the nevirapine-based antiretroviral therapy at study randomization. The authors used ultra-deep pyrosequencing in plasma and long-lived cells to determine nevirapine resistance frequencies. High-frequency nevirapine resistance (defined as ≥ 20% present), but not low-frequency resistance (defined as 1% – 19% present) in plasma, was found to be statistically significantly associated with virologic failure at 24 weeks and 52 weeks. Cellular nevirapine resistance was not associated with virologic failure at either time point.

Persistence of Resistance and Treatment Outcomes in Women After Single-Dose Nevirapine

Boltz and colleagues used allele-specific polymerase chain reaction to detect low-frequency NNRTI-resistant variants at baseline among HIV-infected women who received prior single-dose nevirapine and initiated antiretroviral therapy in the OCTANE (Optimal Combination Therapy After Nevirapine Exposure) trial 1/A5208 (Abstract 154). This randomized study showed higher rates of virologic failure or death in women with prior nevirapine exposure than in those receiving lopinavir/r (26% vs 8%, respectively; $P = .0004$). The authors identified NNRTI-resistant variants in 70 (35%) women who had no mutations detected by standard genotype. Among these 70 women, a higher proportion in the nevirapine-treated group experienced virologic failure or death than in the lopinavir/r group (32% vs 9%; $P = .04$), and this difference was seen across the range of mutants detected (0.3% to > 30%).

Single-dose nevirapine continues to be used widely for PMTCT in RLS despite the high frequency of emergence of antiretroviral drug resistance mutations in women shortly after drug exposure. Yang and colleagues studied the effect of HIV-1 subtypes on persistence of NNRTI resistance mutations in 330 women exposed to single-dose nevirapine in Kenya, Thailand, and Zambia (Abstract 912). None of these women received any additional antiretroviral therapy after the single-dose nevirapine treatment.

Conventional sequencing analysis involving the reverse transcriptase codons 1 to 251 was used to detect NNRTI resistance mutations, whereas allele-specific RT-PCR was employed to identify minor strains of NNRTI resistance mutations, including K103N, V106M/I, Y181C, and G190A. Phylogenetic analysis identified HIV-1 subtype-C infection in 181 women, subtype CRF01_AE infection in 80 women, subtype-A infection in 46 women, and other subtype infections in 23 women. No statistically significant difference in the prevalence of NNRTI resistance mutations using conventional sequencing analysis was seen by HIV-1 subtype. However, resistance mutations as determined by allele-specific RT-PCR were more prevalent in women with subtype-C infection than in those with nonsubtype-C infection, particularly among women exposed to single-dose nevirapine more than 12 months earlier (21% vs 1%; $P < .01$). The authors suggested that NNRTI drug resistance mutations might endure for a longer period of time in subtype-C infections than in nonsubtype-C infections.

Hudelson and colleagues investigated risk factors associated with the emergence and persistence of nevirapine-resistant HIV in breast milk in 51 HIV-infected Ugandan women who had been exposed to single-dose nevirapine for PMTCT (Abstract 913). None of the women received any other antiretroviral therapy. Breast milk and plasma samples were collected and tested at 4 weeks postpartum, with follow-up specimens available for a subset of women. Standard genotyping was successful for 10 breast milk samples with plasma HIV RNA levels above 500 copies/mL and for 21 of 41 samples

with levels below 500 copies/mL; 1 woman was excluded from the analysis. Phylogenetic analysis showed a predominance of subtype-A and -D HIV. Twelve (40%) of 30 breast milk samples had at least 1 nevirapine resistance mutation. The most common mutations were K103N and Y181C. Nevirapine resistance was present by 10 weeks postpartum in 4 of 10 breast milk samples that were available at this time point. The study was limited by a small sample size.

A limited number of studies to date have evaluated the long-term clinical outcomes of antiretroviral therapy among women with previous intrapartum exposure to nevirapine for PMTCT. Chintu and colleagues compared post–12-month clinical outcomes among 5172 Zambian women starting a NNRTI-based regimen: 596 (12%) had prior nevirapine exposure for PMTCT, and 4576 (88%) had no prior exposure (Abstract 914). The following outcome variables were assessed after 12 months of follow-up using Kaplan-Meier analysis: mortality, clinical treatment failure (defined as worsening WHO clinical staging after 3 months of antiretroviral therapy initiation, CD4+ cell count decrease below 95% of pre–antiretroviral therapy initiation level after at least 3 months of antiretroviral therapy [with or without a switch to second-line antiretroviral regimen]), and a composite of the first 2 outcomes.

Baseline characteristics at time of antiretroviral therapy initiation differed between the 2 groups: women with prior nevirapine exposure were younger, had higher baseline CD4+ cell counts, were more likely to have WHO clinical stage 1 or 2 HIV disease, and had higher hemoglobin levels than did women with no previous exposure. Median follow-up was 29 months. A trend toward increased risk of clinical treatment failure did not reach statistical significance (adjusted HR, 1.18; 95% CI, 0.95 – 1.47), and surprisingly, decreased mortality (adjusted HR, 0.53; 95% CI, 0.27 – 1.06) was noted among women with previous nevirapine exposure. The authors cautioned that the finding of a statistically nonsignificant association between prior nevirapine exposure and increased survival might be related to residual confounding.

Virologic failure among HIV-infected South African women exposed to single-dose nevirapine was compared with that among those with no prior exposure in the CIPRA-SA study (Abstract 915). CIPRA-SA was a randomized comparative trial evaluating treatment outcomes of doctor-initiated and -monitored antiretroviral therapy versus doctor-initiated and nurse-monitored antiretroviral therapy in 573 HIV-infected women with baseline CD4+ cell counts below 350/μL. Of 573 women, 165 (29%) previously received single-dose nevirapine, and 29 (5%) received single-dose nevirapine in combination with zidovudine. A majority of the women (89%) received a NNRTI-based regimen. The only baseline characteristic that differed statistically significantly between nevirapine-exposed and -unexposed women was age, with women in the nevirapine-exposure group being younger.

Overall, virologic failure was seen in 9.8% (50 of 511) of the women, with no statistically significant difference between nevirapine-exposed and -unexposed women (12.9% vs 8.2%, respectively). A 2.3-fold higher risk of early virologic failure was seen in nevirapine-exposed women compared with -unexposed women. No statistically significant difference in late virologic failure was observed between the 2 groups of women. Resistance mutations were absent in 32 of 50 resistance tests (64%). Among those with resistance mutations, the following mutations were detected: K103N, K103R, E138A, and M46L.

Weidle and colleagues examined the prevalence of mutations associated with nevirapine resistance among women exposed to single-dose nevirapine more than 1 year before initiating NNRTI-based antiretroviral therapy and assessed whether baseline resistance mutations were associated with treatment failure (Abstract 916). A total of 878 women in Zambia, Thailand, and Kenya were enrolled in the parent study; 355 women had previous nevirapine exposure and 523 did not. A subset of 172 women who started treatment with a NNRTI-based regimen more than 1 year after single-dose nevirapine exposure was included in the analysis. A majority of these women (95%) received nevirapine-based antiretroviral therapy, with the remainder receiving efavirenz-based antiretroviral therapy. Infections with HIV-1 subtypes C and CRF01_AE were common. Median time from single-dose nevirapine exposure to commencement of antiretroviral therapy was 25 months. To screen for specific mutations, investigators used both conventional sequencing involving reverse transcriptase codons 1 to 251 and allele-specific RT-PCR. At baseline, NNRTI resistance mutations were detected in 13% (19 of 152 subjects) of available samples by RT-PCR and in 2% (4 of 163) of available samples by conventional sequencing analysis.

The authors did not find any statistically significant association between detection of nevirapine resistance mutations by RT-PCR and virologic failure (defined as plasma HIV-1 RNA level > 400 copies/mL at 24 or 48 weeks after antiretroviral therapy initiation). Similarly, in subgroup analysis restricted to women with subtype-C HIV infection, no association between these 2 factors was observed. The study did not evaluate the relationship between nevirapine resistance mutations and long-term clinical outcomes such as mortality.

HIV Drug Resistance in Breastfeeding Infants Exposed to Antiretroviral Therapy

Dross and colleagues evaluated the incidence of nevirapine resistance in Mozambique in infants infected with HIV through breastfeeding (Abstract 917). The prospective observational study involved 740 infants who received single-dose nevirapine for PMTCT. Nevirapine resistance was found in 7 (47%) of 15 infants who acquired HIV. The majority of the infants with nevirapine resistance (86%) had 100% mutant HIV populations in their first positive DNA PCR test specimen, which persisted through 3 months to 12 months of age. One

infant with nevirapine resistance had 20% mutant populations at Y181C, and none had the V106M mutant detected.

Lidstrom and colleagues compared nevirapine resistance in infants who were HIV infected by 14 weeks of age and received extended nevirapine treatment either with or without zidovudine prophylaxis in the PEPI–Malawi (Post-Exposure Prophylaxis for Infants–Malawi) study (Abstract 918). The PEPI–Malawi study was a randomized controlled trial in which infants received 1 of the following regimens at birth for PMTCT: a control regimen consisting of single-dose nevirapine plus 1 week of daily zidovudine; 1 week of daily zidovudine plus extended nevirapine daily to age 14 weeks; or extended nevirapine plus zidovudine to age 14 weeks. The study showed that the risk of HIV transmission at 9 months of age was lower in both extended-prophylaxis groups than in the control group; HIV transmission risk in the 2 extended-prophylaxis groups showed no statistically significant difference.

In the current analysis, the authors compared nevirapine resistance in 150 HIV-infected infants receiving extended nevirapine with those receiving extended nevirapine plus zidovudine using HIV genotyping assays from plasma specimens collected at 14 weeks of age. Nevirapine resistance was lower in the extended-nevirapine plus zidovudine group than in the extended-nevirapine group (65.6% vs 86.0%; $P = .01$). In subgroup analyses, this difference was observed only in a subset of infants with in utero HIV infection who stopped prophylaxis by 6 weeks of age. None of the samples had any detectable zidovudine resistance mutations.

A shift in nevirapine resistance from low to high frequencies was found in Ethiopian infants for whom extended nevirapine prophylaxis was failing for the prevention of breast-milk transmission of HIV in the SWEN trial (Abstract 919). HIV genotyping was performed on dried blood spots collected at 6 months in 53 HIV-infected infants, most of whom (81%) had their HIV infection diagnosed at or before 14 weeks of age, with the remainder of infections diagnosed by 6 months of age. High-frequency nevirapine resistance was defined as nevirapine resistance detected by population genotyping, whereas low-frequency resistance was defined as nevirapine resistance detected only through cloning.

The authors observed that infants receiving extended nevirapine who became HIV infected by 14 weeks of age had a greater prevalence of high-frequency nevirapine resistance at 6 months than did infants receiving single-dose nevirapine (62% vs 18%, respectively; $P = .05$). However, when both high- and low-frequency nevirapine resistance were included in the analysis, there was no statistically significant difference in the prevalence of nevirapine resistance between the 2 groups. Among infants who received diagnoses of HIV infection after 14 weeks of age, the prevalence of high- and low-frequency nevirapine resistance was similar in the 2 groups.

Antiretroviral Regimens, Viral Response, and Mother-to-Child Transmission Outcomes

Tariq and colleagues investigated the impact of non–zidovudine-containing antiretroviral therapy on pregnancy and clinical outcomes using data from a population-based surveillance study and a multicenter cohort study in 10 European countries (Abstract 895). HIV-infected women who had at least 14 days of antiretroviral therapy in pregnancy and had live, singleton births were included in the analysis. Sixteen percent of the women received non–zidovudine-containing antiretroviral therapy, with tenofovir and abacavir the most common alternate nRTIs. There was no statistically significant difference in the following outcome variables between women who received zidovudine-containing antiretroviral therapy and those who received non–zidovudine-containing antiretroviral therapy in multivariate models: detectable maternal viral load at delivery, risk of congenital abnormality among infants born to all women, risk of congenital abnormality among infants born to women exposed to antiretroviral therapy during the first trimester, and risk of mother-to-child transmission of HIV.

Briand and colleagues found no negative impact of prior short-term antiretroviral prophylaxis for PMTCT on virologic response to PI-based antiretroviral therapy in subsequent pregnancies among HIV-infected women (Abstract 898). Data from the French Perinatal Cohort, a multicenter prospective cohort study in France, were analyzed. At onset of last pregnancy, 714 women were naive to antiretroviral therapy, and 193 previously received antiretroviral therapy for PMTCT. Among women who received antiretroviral therapy during their most recent pregnancy, 43% had a PI-based regimen, 5% a non–PI-based regimen, 19% dual-nRTI therapy, and 33% zidovudine monotherapy. A majority of the women (89%) were started on a PI-based regimen during their subsequent pregnancy. Among these women who received PI-based antiretroviral therapy during their subsequent pregnancy, there was no statistically significant difference in the proportion achieving virologic suppression (plasma HIV RNA level < 50 copies/mL) at delivery between those who were naive to antiretroviral therapy and those who previously received antiretroviral therapy.

Tuberculosis Impact on Prevention of Mother-to-Child Transmission of HIV

Gupta and colleagues noted an association between maternal active TB and MTCT of HIV among 783 mother-infant pairs enrolled in the India SWEN study (Abstract 899). The SWEN study was a comparative trial of extended nevirapine treatment versus single-dose nevirapine for PMTCT of HIV in breastfeeding infants. Median follow-up was 1 year. Three mothers had active TB diagnosed in pregnancy (prevalent TB), and 30 mothers had active TB by 12 months postpartum (incident TB). In multivariate analysis, mothers with prevalent or incident TB were 2.53 times more likely to transmit HIV to their infants than were mothers without active TB, after adjusting for maternal and child factors such as

maternal HIV viral load, maternal antiretroviral therapy, infant nevirapine prophylaxis, and duration of breastfeeding. The study findings were limited by lack of culture confirmation in all TB cases and lack of ascertainment of potential confounders like HCV coinfection and nutritional status.

Breast-Milk Shedding of HIV and Antiretroviral Therapy Impact

Exclusive breastfeeding in the first 6 months of life poses a risk of 4% for MTCT of HIV, followed by a risk of 1% per month after 6 months.[8] Neveu and colleagues conducted a nested, case-control study among HIV-infected women and their infants in KwaZulu-Natal, South Africa, to examine the relationship between HIV RNA shedding in breast milk, cumulative HIV exposure through breast milk, and postnatal HIV transmission (Abstract 901). Infants who acquired HIV from their HIV-infected mothers between 6 weeks and 200 days of age were considered cases, and HIV-uninfected infants born to HIV-infected mothers served as control subjects. A total of 36 mother-infant pairs with complete data were included. Breast milk and blood specimens were collected monthly. The authors observed that the cases were more likely to shed HIV-1 RNA in breast milk than were the control subjects. In addition, cumulative breast milk HIV-1 RNA exposure (calculated between 6 weeks and time of HIV acquisition) was 15-fold higher in cases than in control subjects. The association between cumulative breast milk HIV RNA exposure and postnatal HIV transmission remained statistically significant after controlling for maternal antenatal CD4+ T-cell count and plasma viral load.

Treatment of HIV Infection in Children

Response to Initial Antiretroviral Therapy in Children

Violari and colleagues studied early virologic and immunologic outcomes in 386 HIV-infected infants initiating antiretroviral therapy as part of the CHER (Children With HIV Early Antiretroviral Therapy) study in South Africa (Abstract 843). The children were started on a lopinavir/r-based regimen at a median age of 8.4 weeks. Virologic and immunologic responses were assessed at 24 weeks and 40 (or 48) weeks after initiating antiretroviral therapy. Virologic suppression was defined as plasma HIV RNA level below 400 copies/mL. An increase in CD4+ percentage to a level that was at least 10% above the baseline pre–antiretroviral-therapy level constituted an immunologic response.

At 24 weeks, virologic suppression was seen in 71% of children and in 77% at 40 or 48 weeks after antiretroviral therapy initiation, with a minority (8%) experiencing virologic rebound to plasma HIV RNA levels above 400 copies/mL at 40 or 48 weeks after having achieved virologic suppression at 24 weeks. Virologic suppression was not associated with age at antiretroviral therapy commencement, weight-for-age *z* score, viral load, or sex. However, children with active TB diagnosed before or within 1 month of starting antiretroviral therapy and receiving concurrent TB treatment had poorer virologic response at 24 weeks than did children without TB coinfections. The median increase in CD4+ percentage from baseline level at the time of antiretroviral therapy initiation was 7% at 24 weeks and 40 or 48 weeks. Immunologic response was associated with lower baseline CD4+ percentage.

Raltegravir in Children

Nachman and colleagues presented interim pharmacokinetics, 12-week efficacy, and safety results from IMPAACT P1066 (International Maternal Pediatric Adolescent AIDS Clinical Trials P1066), an open-label study of raltegravir in HIV-infected, treatment-experienced children (Abstract 161LB). This analysis included only 10 children between the ages of 6 years and 11 years from the cohort in which an oral chewable tablet formulation of raltegravir was administered. All children had plasma HIV RNA levels above 1000 copies/mL at baseline and were naive to raltegravir. Median baseline CD4+ cell count was 456/μL and plasma HIV RNA level was 4.2 $\log_{10}$ copies/mL. The authors found lower pharmacokinetic variability and lower drug clearance among children receiving the oral chewable tablet formulation than in children in the other cohort, who received an adult formulation of raltegravir. Seven of the 10 children receiving oral chewable tablets had plasma HIV RNA levels suppressed below 400 copies/mL at 12 weeks.

HIV Drug Resistance after Treatment Failure in Children

Three studies evaluated antiretroviral drug resistance after treatment failure in children. Limited access to virologic testing in RLS has led to an increased risk of unrecognized virologic failure and emergence of drug resistance. Achan and colleagues investigated the incidence of early virologic failure and evolution of drug resistance mutations in 126 Ugandan children starting antiretroviral therapy as part of the CHAMP (Children with HIV and Malaria Project) (Abstract 849). The authors defined virologic failure as plasma HIV RNA level above 1000 copies/mL in the 6-month to 9-month period following antiretroviral therapy initiation, and they excluded data from children with antiretroviral therapy nonadherence. Initial antiretroviral therapy consisted of nevirapine plus either zidovudine/lamivudine or stavudine plus lamivudine. Median follow-up was 746 days beyond 6 months of antiretroviral therapy. Eighteen children (14%) developed early virologic failure. All of these children had persistent, detectable viremia during the follow-up period. Only 2 of these children had mothers who used nevirapine for PMTCT. Within the first 6 months, M184V and NNRTI RAMs emerged in most children, thymidine analogue–associated mutations (TAMs) emerged after 12 months, and 2 etravirine-associated mutations emerged by 30 months.

Another study on drug resistance in HIV-infected children in Uganda showed higher rates of virologic failure

in children exposed to single-dose nevirapine for PMTCT than in unexposed children (Abstract 850). At 24 weeks after antiretroviral therapy initiation, 65.4% (17 of 26) of children exposed to single-dose nevirapine experienced virologic failure (defined as plasma HIV RNA level > 400 copies/mL) compared with 36.5% (19 of 52) of unexposed children (P = .0127). Similar rates of virologic failure were seen at 48 weeks: 62.1% (18 of 29) of exposed children compared with 32.1% (17 of 53) of unexposed children (P = .0086). NNRTI- and nRTI-associated resistance mutations were analyzed in a subset of 20 children (15 exposed and 5 unexposed). Among children exposed to single-dose nevirapine, the most common NNRTI resistance mutations were Y181C, G190A/G, K103N, and V108I/V, and the most frequent nRTI resistance mutations were M184V, D67N, and K70R. Unexposed children had fewer drug resistance mutations than exposed children did; the predominant mutations were K103N and M184V. It is important to note that only subtype-A and subtype-D HIV-1 strains were represented in this drug resistance analysis.

Darunavir-associated resistance mutations in PI-naive and -experienced HIV-infected children in the United Kingdom were examined using combined data from the CHIPS (Collaborative HIV Paediatric Study) and the UK HIV Drug Resistance Database (Abstract 851). Darunavir resistance mutations were derived from the 2008 IAS–USA mutations list[9] and the Stanford University Drug Resistance Database.[10] Among 344 PI-naive children, 14 (3%) were found to have a single mutation, and no children had more than 1 mutation. A majority of the PI-naive children with a single mutation (83%) had nonsubtype-B HIV-1 infection. A total of 156 PI-experienced children were studied. Median time on PI-based antiretroviral therapy was 2.6 years, with approximately one-third of the children receiving lopinavir/r as their only PI. Of the PI-experienced children, 21 (13%) had a single mutation, 5 (3%) had 2 mutations, and 3 (2%) had 3 mutations. Intermediate-level resistance to darunavir, as determined by the Stanford University database, was observed in only 3 (2%) PI-experienced children.

On multivariate analysis, the following factors were associated with increased number of darunavir resistance mutations: longer time taking PI-based antiretroviral therapy, prior exposure to PIs other than lopinavir/r, and larger area under the viremia curve from PI initiation. The authors suggested that given the low prevalence of darunavir-associated resistance mutations in PI-naive and PI-experienced children in the study, darunavir/r could be used as an initial or a second-line PI-based regimen.

Survival of HIV-Infected Children in Africa

Becquet and colleagues from the UNAIDS Child Survival Working Group conducted a pooled analysis of 12 trials and cohort studies in sub-Saharan Africa to estimate survival of children infected with HIV perinatally or through breastfeeding (Abstract 840). Estimations of mortality were based on the HIV serostatus of the child, with timing of HIV acquisition incorporated into the mortality calculations for HIV-infected children. Random-effects Weibull regression models were used, with adjustments for sub-Saharan African region, maternal vital status, maternal CD4+ cell count, any breastfeeding, and HIV serostatus and sex of the child. Non–HIV-related causes of mortality were excluded from the models. A total of 2509 HIV-infected and 8964 uninfected children were analyzed. Overall estimated 24-month mortality was higher among HIV-infected children than among uninfected children, regardless of timing of HIV acquisition. Kaplan-Meier survival analysis showed that among HIV-infected children, those with HIV acquired during breastfeeding had statistically significantly lower estimated 18-month mortality than did children infected with HIV perinatally (36% vs 60%, respectively).

Venkatesh and colleagues examined correlates of infant morbidity and mortality within the first 100 days of life in a South African multicenter prospective study of infants who were born to HIV-infected women and received antiretroviral drugs for postexposure prophylaxis (Abstract 841). A total of 848 HIV-infected mother and infant pairs were included in the analysis. Overall, the 100-day cumulative probability of not having a serious adverse event (defined as infant hospitalization) was .89 (95% CI, 0.85 – 0.92), and the cumulative probability of survival was .98 (95% CI, 0.97 – 0.99). Gastrointestinal and respiratory infections were the most frequent causes of infant morbidity. Hospitalization occurred more frequently among HIV-infected than -uninfected infants and among those with maternal plasma HIV RNA level above 100,000 copies/mL; maternal age below 20 years was associated with less frequent hospitalization. Not surprisingly, statistically significant predictors of mortality were infant HIV infection (HR, 4.10; 95% CI, 1.18 – 14.31) and maternal plasma HIV RNA level above 100,000 copies/mL (HR, 6.93; 95% CI, 1.64 – 29.26). The authors did not find infant feeding route (ie, breastfeeding vs formula feeding) to be associated with infant morbidity or mortality. The authors recommended prompt initiation of antiretroviral therapy in HIV-infected women of childbearing age, given the increased risk of hospitalization and mortality among infants whose mothers had plasma HIV RNA levels above 100,000 copies/mL.

Infant Outcome after Prenatal Antiretroviral Therapy Exposure

Conway and colleagues examined the prevalence of congenital abnormalities among 1112 infants with in utero exposure to antiretroviral therapy using data from IMPAACT P1025, a large prospective cohort study in the United States (Abstract 923). Sixty-one children were found to have congenital anomalies (as defined by The Antiretroviral Pregnancy Registry, www.apregistry.com), yielding an anomaly rate of 5.49 per 100 live births (compared with 2.8/100 live births among the general population and 3.56/100 live births among HIV-infected women and their children). Exposure to efavi-

renz during the first trimester had a 2.89-fold increased risk of congenital anomalies, whereas exposure to nonefavirenz antiretroviral therapy in any trimester during pregnancy was not associated with increased anomaly risk. Other factors such as race or ethnicity, maternal age, folate supplementation, and in utero exposure to alcohol, tobacco, heroin, and other illicit drugs were not associated with increased anomaly risk.

In another study, no increased risk of congenital, renal, or growth abnormalities was found with in utero exposure to tenofovir among infants born to HIV-infected mothers in a subset of the DART study in Uganda and Zimbabwe (Abstract 924). A smaller comparative study among 40 children with and without in utero exposure to tenofovir did not show any elevation in cystatin C or urea levels in either group during the 2-year follow-up period (Abstract 925). The effects of in utero exposure to tenofovir, particularly during the second and third trimesters, on bone development and metabolism of HIV-uninfected children born to HIV-infected mothers were examined by Vigano and colleagues (Abstract 926). No statistically significant differences in anthropometric parameters, tibial speed of sound *z* score (as measured by quantitative ultrasound), and levels of serum bone alkaline phosphatase and C-terminal telopeptide of type I collagen were noted between children with in utero tenofovir exposure and those without such drug exposure.

Antiretroviral Therapy During Treatment for Tuberculosis

Lopinavir/r is superior to an NNRTI-based regimen as initial therapy in HIV-infected infants and young children previously exposed to nevirapine for PMTCT of HIV.[11] However, modifications to the antiretroviral regimen are necessary in HIV and TB coinfections because of drug interactions between lopinavir/r and rifampin. Moodley and colleagues conducted a retrospective case-control study evaluating virologic outcomes in South African HIV-infected children who started PI-based antiretroviral therapy (Abstract 160). A total of 526 children were included in the study: 294 children who were treated concurrently with TB drugs (cases) and 232 children who did not receive TB treatment (control subjects). All control subjects were treated with a standard lopinavir/r-based regimen. The cases were stratified according to their PI combinations: (1) super-boosted lopinavir/r (ritonavir concentrations were increased up to 1:1 concentration with lopinavir by the addition of ritonavir), (2) double-dose lopinavir/r (standard doses of lopinavir/r were doubled), and (3) ritonavir alone. All children received stavudine plus lamivudine.

The authors found that a lower proportion of children treated with double-dose lopinavir/r or ritonavir had viral suppression at 6 months than that of control subjects. At 12 months the proportion of children receiving double-dose lopinavir/r or super-boosted lopinavir/r who had suppressed HIV viral loads was similar to that of the control subjects (76.9% and 82.9% vs 83.3%, respectively). However, children taking ritonavir alone were less likely to have viral suppression than were control subjects at 12 months (63.9% vs 83.3%, respectively). Not surprisingly, severe elevations of alanine transaminase levels were most common in children who received super-boosted lopinavir/r, occurring in nearly 30% of the children. The authors cautioned against use of ritonavir alone with concurrent rifampin-based TB treatment and suggested that super-boosted lopinavir/r was best. The authors did not explore the reason for the worse 6-month outcome in children taking double-dose lopinavir/r than in children taking super-boosted lopinavir/r.

Resistance

Prevalence and Consequences of Transmitted Drug Resistance

Two studies examined the prevalence of transmitted drug resistance associated mutations (TDRM) in newly diagnosed HIV-infected persons from the US HIV surveillance system. The surveillance system collects population-based drug resistance data on all new HIV diagnoses from 10 states and 1 county in the United States. Kim and colleagues analyzed the prevalence of TDRM among new HIV diagnoses in 2007 using the WHO mutation list (Abstract 580).[12] A total of 10,496 new HIV diagnoses occurred in 2007, one-fourth of whom had genomic sequences reported to the national surveillance list. Sixteen percent of the new HIV diagnoses with available genomic sequences had at least 1 TDRM. Eight percent had NNRTI-associated resistance mutations, 6% had nRTI resistance mutations, and 4% had PI resistance mutations. Prevalence of multiclass resistance was low: 2% for 2 drug classes and less than 1% for 3 drug classes. The presence of TDRM was not associated with age, sex, race, or transmission mode.

Prejean and colleagues compared the prevalence of TDRM between recent and longstanding HIV infections using data from the US HIV surveillance system (Abstract 581). The surveillance system utilizes a serologic testing algorithm and the BED HIV-1 capture enzyme immunoassay to distinguish recent from longstanding HIV infections among persons with newly diagnosed HIV infection. A total of 1626 antiretroviral-naive persons with newly diagnosed HIV infection in 2006 were included in the analysis. A majority (73.1%) had longstanding HIV infections or AIDS within 6 months of HIV diagnosis. The remainder (26.9%) had recent HIV infections. Prevalence of TDRM was higher in recently infected persons than in those with longstanding infections (18.3% vs 13.8%; $P = .02$). TDRM related to NNRTIs and nRTIs were seen more frequently in persons with recent infections than in those with longstanding infections. No differences in the prevalence of PI-associated resistance mutations were seen between the 2 groups. This study confirmed findings from previous studies noting a higher prevalence of TDRM in recent infections than in longstanding infections, reflecting either increasing transmission of drug-resistant HIV or a propensity of HIV strains to revert to wild type over time

with archiving of low-frequency mutant viral populations.

Changing Prevalence of Multidrug Resistance

Abraham and colleagues applied novel multiple imputation methodology to estimate the cumulative prevalence of multiclass drug resistance from 2000 to 2005 among 9786 HIV-infected persons from 9 HIV cohorts within the NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design) consortium (Abstract 584). HIV-infected persons receiving antiretroviral therapy who had no known drug resistance before antiretroviral therapy initiation and who had at least 1 study visit that included a viral load result were included in the analysis. Genotypic test results were available for nearly one-third of the sample. The Stanford University HIV Drug Resistance Database[9] was used to determine drug resistance. A steady decline was observed in the prevalence of multiclass resistance among those with available genotypic test results from 2000 to 2005, from 57.9% in 2000 to 38.5% in 2005 for resistance to at least 2 drug classes and from 22.9% in 2000 to 19.0% in 2005 for resistance to 3 drug classes. Using imputation methodology to estimate accumulated drug resistance among those without genotypic testing results, the authors noted an increase in the prevalence of multiclass resistance during the study period, from 13.9% in 2000 to 18.8% in 2005 for accumulated resistance to at least 2 classes and from 5.8% in 2000 to 8.6% in 2005 for accumulated resistance to 3 classes.

Aldous and colleagues noted a decline in the prevalence of drug resistance mutations from 2002 to 2008 in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems Cohort from 6 sites in the United States (Abstract 585). A subset of persons taking antiretroviral therapy who had available genotypic testing results was analyzed. The 2008 IAS–USA criteria[8] were used to screen for major and minor drug resistance mutations. A steady decrease in the prevalence of at least 1 major PI resistance mutation, at least 1 thymidine analogue resistance mutation, and the M184V mutation was observed over the 7-year period. The pattern for the prevalence of at least 1 major NNRTI resistance mutation was slightly different over time: it was 45% in 2002, increased to 52% in 2004, and gradually dropped to 36% by 2008. A steady decline in multiclass resistance was also noted.

Sequencing Technologies

New sequencing technologies were a major focus of this year's conference. An oral presentation by Henn and colleagues provided an overview and evaluation of deep-sequencing technologies (Abstract 9). New platforms for deep sequencing (also known as ultra-deep sequencing and pyrosequencing) have reduced costs and enabled high-throughput, high-sensitivity analyses. In the presentation, different proprietary deep-sequencing assays were compared in terms of read-length and throughput. As opposed to "bulk-sequencing" assays that produce a genotypic analysis of the majority population in a sample, deep sequencing was described as analogous to single-genome amplification. The assays utilize capture technologies, such as beads or wells, to allow the amplification of single DNA molecules by PCR. The advantages of deep sequencing include its ability to sequence unknown viral elements, characterize quasi species within an individual, and evaluate diversity across the entire viral genome. Another excellent overview of deep-sequencing technology was presented by Brumme, the moderator of Session 15.

Resistance to Nucleotide Analogue Reverse Transcriptase Inhibitors

Coutsinos and colleagues examined the mechanisms responsible for the emergence of the K65R mutation in subtype-B and subtype-C HIV (Abstract 548). Referring to prior work, the authors note that subtype-C HIV-1 is more prone to the emergence of the K65R mutational pathway than is subtype-B HIV. Nucleotide extension assays of the *pol* gene were performed utilizing recombinant subtype-B and subtype-C HIV-1 reverse transcriptase (RT). An enzymatic pause at the K65R substitution site was observed in subtype-C virus. Incorrect nucleotide incorporation via dislocation mutagenesis was the postulated mechanism. This observation has implications for the introduction of tenofovir for initial antiretroviral therapy in regions where subtype-C virus is endemic.

A related study by Varghese examined the frequency of minority K65R variants in subtype-B and subtype-C virus using ultra-deep sequencing (Abstract 547). Antiretroviral therapy–naive subjects with subtype-B and subtype-C HIV were compared for the presence of K65R minority populations. K65R was detected in 0.85% of subtype-C and 0.25% of subtype-B samples (P = .01). However, the authors noted that these minority variants may be spurious in subtype-C HIV and that ultra-deep sequencing may overestimate the clinical relevance of this mutation.

Resistance to Nonnucleoside Analogue Reverse Transcriptase Inhibitors

The data on NNRTI resistance presented at the conference focused primarily on etravirine RAMs. Asahchop and colleagues conducted in vitro etravirine RAM selection assays in different HIV-1 subtypes (Abstract 552). Subtype-B, subtype-C, and CRF02_AG clones underwent serial passage through increasing concentrations of etravirine and efavirenz. After 18 weeks of etravirine selective drug pressure, the E138K substitution was the first to emerge in the 3 subtypes tested. No differences in RAMs selected were observed between the different subtypes. The CRF02_AG clone that harbored only the E138K mutation showed a 5.3-fold increase in the 50% inhibitory concentration (IC_{50}) to etravirine. Efavirenz did not select for this mutation.

Maiga and colleagues examined the susceptibility to etravirine in isolates from nonsubtype-B HIV-1-infected patients (Abstract 553). Isolates from 726

antiretroviral therapy–naive individuals from France and Mali underwent genotypic and phenotypic evaluation. The most common etravirine RAMs in this treatment-naive population were V90I, E138A, V106I, and V179E. Overall, 10.3% of patients had virus with at least 1 etravirine RAM: 9.8% had 1 etravirine RAM, 0.5% had 2 etravirine RAMs, and none had 3 or more RAMs. Only the CRF02_AG strain harbored more than 1 etravirine RAM ($P = .004$ compared with all other subtypes). The following mutational combinations were associated with an increased IC_{50} fold increase to etravirine: E138A plus V179I (fold change, 5.2), Y181C plus H221Y (fold change, 11.1), V90I plus Y181C (fold change, 3.3).

Resistance to Protease Inhibitors

Koh and colleagues presented mechanistic data on darunavir and tipranavir resistance mechanisms (Abstract 559). Both darunavir and tipranavir block protease activity by inhibition of protease subunit dimerization, which is required for protease's catalytic activity. The authors looked at the ability of wild-type and mutant protease to dimerize in the presence or absence of these drugs. Fluorescence resonance energy transfer, a florescent-tag–based system used to detect dimerization on the basis of color change, was used. Only the combined presence of the major darunavir resistance mutations V32I, L33F, I54M, and I84V was associated with an inability of darunavir to block protease dimerization in vitro. In contrast, tipranavir failed to block dimerization in the presence of any of the major mutations L24M, L33I, or L33F. This study confirms the high genetic barrier to darunavir resistance and provides a mechanistic explanation for this barrier.

Parry and colleagues presented data on the compensatory mechanisms of HIV-1 in maintaining replication fitness despite the accumulation of protease mutations (Abstract 561). The authors describe mutational pathways in the *gag* sequence encoding for matrix protein. These mutations were able to restore normal replication capacity in highly replication-capacity-deficient (replication capacity, 5%) protease-resistant clones. The following compensatory mutations in *gag* matrix domain were associated with the restoration of viral replication capacity: R76K, Y79F, and T81A.

CC Chemokine Receptor Tropism and CCR5 Antagonists

A substantial amount of information was presented at the conference on the ability to predict HIV-1 coreceptor tropism and CCR5 antagonist antiviral activity using novel technologies. These new technologies include deep sequencing of the V3 loop of the *env* gene and the amplification of viral genomic sequences from nonplasma sources such as proviral and episomal DNA. An entire session was dedicated to the use of new technologies in the prediction of HIV-1 coreceptor use (Session 15). The following section is divided into thematic subsections, reflecting the volume of data presented on this topic.

Comparisons of tropism assays. Several presentations compared the predictive capacity of different tropism assays. These included comparisons of the 2 commercially available phenotypic tropism assays, the enhanced-sensitivity and the older tropism assay (both Monogram Biosciences, Inc, South San Francisco, CA), as well as evaluation of genotypic predictions of tropism.

Wilkin and colleagues reexamined tropism data from cohorts and clinical trials using the enhanced-sensitivity tropism assay rather than the original tropism assay (Abstract 538). The enhanced-sensitivity tropism assay and the original tropism assay are described as having a sensitivity of 100% to detect non–R5-tropic virus at a population prevalence of 0.3% and 5%, respectively. Samples from participants in the CPCRA (Community Program for Clinical Research on AIDS) cohort, and the New Works Concept Sheet 261, MERIT (Maraviroc versus Efavirenz Regimens as Initial Therapy), and ACTG 5211 clinical trials were included in the analysis. The outcome of interest was the proportion of patients with virus reclassified from having R5 tropism to having dual-mixed, X4 (DM/X4) tropism. Of 2407 patients included in the analysis, 84% were treatment naive. On average, the enhanced-sensitivity assay detected between 8% and 13% more DM/X4 tropic virus than did the original tropism assay. One-fourth of treatment-experienced patients with R5-tropic virus as determined by the original assay were found to have virus with DM/X4-tropism by the enhanced-sensitivity assay. In a multivariate analysis, lower CD4+ cell count was the only factor statistically significantly associated with reclassification to DM/X4 tropism.

Use of genotypic analysis in the prediction of coreceptor tropism was the main focus of presentations on this topic. McGovern and colleagues retrospectively used V3-loop sequencing to evaluate maraviroc response in the MERIT trial (Abstract 92). MERIT was a randomized clinical trial comparing the safety and efficacy of maraviroc with those of efavirenz, both coadministered with zidovudine/lamivudine, in treatment-naive patients. R5 tropism as determined by the original tropism assay was an inclusion criterion for the study. In this analysis, the HIV-1 V3 loop was genotyped using population-based sequencing. The analysis was performed with investigators blinded to treatment response and to reanalysis of baseline tropism with the enhanced-sensitivity assay. Utilizing the geno2pheno coreceptor tropism predictor algorithm (http://www.geno2pheno.org/), V3-loop sequencing was highly predictive of response to maraviroc. Patients with virus screened as non–R5-tropic by genotypic testing had virus with a more rapid change in tropism. The authors argue that the enhanced-sensitivity assay and V3-loop genotyping showed equivalence in predicting response to maraviroc. They also commented that population sequencing had a good predictive value of virologic response and that deep sequencing may not be required in clinical practice.

Swenson and colleagues looked at the use of V3-loop deep sequenc-

ing in predicting coreceptor tropism (Abstract 545). The authors retrospectively sequenced samples obtained from participants in 4 maraviroc clinical trials: MERIT, MOTIVATE-1 and -2 (Maraviroc Versus Optimized Therapy in Viremic Antiretroviral Treatment–Experienced Patients 1 and 2), and A4001029 (the MERIT trial is described above; MOTIVATE-1 and -2 were randomized controlled trials of maraviroc in treatment-experienced patients; and A4001029 was a trial of maraviroc in non–R5-tropic infections). All trials originally used the older tropism assay to determine tropism for the inclusion or exclusion criterion.

In this analysis, deep-sequencing results were compared with results from the original phenotypic assay. In the deep-sequence analysis, tropism was designated R5 if less than 2% of viral sequences were determined to be X4 tropic by the geno2pheno algorithm. There was good correlation between results of the V3 loop deep-sequencing assay and phenotypic prediction by the original tropism assay. In addition, baseline samples from the MERIT trial were also reanalyzed using the enhanced-sensitivity tropism assay, and these results showed equivalent correlation with V3-loop sequencing. Finally, in the combined analysis of the treatment–experienced trials, samples initially screened as R5 tropic by the original assay were more likely to be reclassified as non-R5 tropic at baseline by deep-sequencing analysis. The authors did not report the proportion of subjects with virus reclassified to DM/X4 tropism in the genotypic analysis.

Pou and colleagues compared deep sequencing analysis to analysis using the enhanced-sensitivity tropism assay and found close correlation between the 2 technologies (Abstract 544). However, deep sequencing had the highest sensitivity for detection of X4-tropic virus in PBMCs compared with plasma. Van't Wout and colleagues compared deep sequencing with phenotypic assay in predicting tropism in a subgroup of subjects in the Amsterdam cohort (Abstract 276). They also found earlier detection of X4-tropic virus using sequencing analysis. Sanchez and colleagues presented a predictive model of X4 tropism in treatment-experienced patients based on clinical and deep-sequencing criteria (Abstract 543).

Tropism prediction using viral DNA sequencing. Soulie and colleagues examined proviral DNA sequences in 140 HIV-1-infected patients taking maximally suppressive antiretroviral therapy (Abstract 537). All patients had plasma HIV-1 RNA levels below 40 copies/mL and all were CCR5-antagonist naive. Coreceptor use was determined with the geno2pheno algorithm. Thirty-one percent of subjects harbored X4-tropic virus. The following predictors of X4 tropism were investigated in a multivariate analysis: time since HIV diagnosis, duration of treatment, length of time with undetectable viral load, HIV subtype, current treatment regimen, number of prior regimens, age, sex, phenotypic sensitivity score, and nadir and current CD4+ cell counts.

Nadir CD4+ cell count was the only variable associated with X4 tropism; the median CD4+ cell count nadir for DM/X4-tropic virus was 108/μL and was 193/μL for R5-tropic virus. However, X4-tropic virus was found in patients at all CD4+ cell count nadir strata. Among patients with the R5-tropic strains, 4.1% harbored maraviroc-associated resistance mutations despite being naive to CCR5 antagonists. The authors cautioned that tropism analysis should be performed in patients taking suppressive antiretroviral therapy before they undergo a switch to CCR5-antagonist-containing regimens, even in patients with high CD4+ cell count nadirs. It is unclear when this technology will become commercially available to clinicians.

Babic and colleagues reported on the use of episomal DNA for V3-loop sequencing (Abstract 540). The authors stated that episomal DNA has the advantage of being dynamic and nonarchival, representing recent infection events. The sample included 14 patients who experienced virologic failure in the vicriviroc trial ACTG A5211. All study participants had virus that was classified R5 tropic by the original tropism assay. PBMCs were collected at various time points, and the full length of *env* was amplified from episomal DNA. V3-loop sequencing from episomal DNA was concordant with the phenotypic tropism assay in 10 of 14 subjects. In 2 subjects there was discordance at all time points, and in 2 subjects DNA sequencing predicted subsequent tropism shift from R5 to DM/X4 at earlier time points than predicted by phenotypic tropism analysis. The authors concluded that episomal DNA sequencing is a useful method that has high concordance with tropism analysis and may be applicable to aviremic patients as well.

Mechanisms of CCR5-antagonist resistance and prevalence of CCR5 coreceptor tropism. Svitcher and colleagues provided data on the genetic determinants of HIV-1 coreceptor use (Abstract 542). Tropism was assessed using the enhanced-sensitivity tropism assay and V3-loop sequencing in samples from 279 patients. The presence of wild-type S11S and the substitutions T22A and E25D of the V3 loop were highly correlated with R5 tropism. Several novel V3-loop genetic mutations were associated with X4 tropism. The authors also show that differential binding affinities of the V3 loop to the CCR5 N-terminus are implicated in the determination of viral coreceptor preference. Yoshimura and colleagues describe a 2-step escape pathway induced by in vitro exposure to maraviroc (Abstract 535). Henrich and colleagues presented data on vicriviroc resistance in subtype-C HIV-1-infected patients (Abstract 534). The authors constructed a series of recombinant viruses and illustrate that several RAMs at the V3 loop are required to produce vicriviroc resistance.

Craig and colleagues presented 96-week data on mechanisms of virologic failure in the MERIT trial (Abstract 536). All patients with DM-tropic virus at baseline by enhanced-sensitivity tropism assay reanalysis were excluded from the current study. Subjects with a HIV-1 RNA level above 500 copies/mL while receiving study therapy were in-

cluded. There were 73 of 311 subjects in the maraviroc group and 43 of 304 subjects in the efavirenz group. In the maraviroc group, 53% had virus that exhibited lamivudine resistance, 26% had maraviroc resistance, and 3% had zidovudine resistance. There were 19 subjects in the maraviroc group experiencing treatment failure without nRTI resistance mutations or maraviroc resistance mutations; of these, 12 had X4-tropic virus and 7 had R5-tropic virus at failure. The specific mutations identified at failure are not described. In the efavirenz group, efavirenz mutations were found in virus of 53% of subjects and lamivudine mutations in 30%. Rates of virologic suppression were similar between the 2 treatment groups.

Lin and colleagues estimated the prevalence of X4-tropic virus among treatment-naive subtype-C HIV-1-infected women in the Mashi study (Abstract 278). The Mashi study examined different strategies for PMTCT in Botswana. Women who reached the CD4+ cell count criterion for starting antiretroviral therapy (< 200/µL) were offered antiretroviral therapy and also underwent tropism analysis. An in-house genotypic tropism assay was performed that was validated to detect minority X4-tropic virus at a population prevalence of 1%. A total of 206 women met criteria for treatment. The median age at enrollment was 29 years, the baseline CD4+ cell count was 132/µL, and the plasma HIV-1 RNA level was 4.95 $\log_{10}$ copies/mL; 51% had previously received single-dose nevirapine for PMTCT. Samples were obtained for 137 women, of which 97% could be genotyped.

Of treatment-naive women in this study, 74% had R5-tropic virus and 26% showed DM virus. No subject had pure X4-tropic virus. The probability of having DM virus was similar across study sites and by original study group. There were no statistically significant differences observed in clinical outcomes between women with R5 virus versus DM virus, although the authors observed a trend toward earlier initiation of antiretroviral therapy and higher mortality in DM- versus R5-tropic subjects.

Jacobson and colleagues evaluated viral resistance and coreceptor tropism in subjects exposed to the investigational drug PRO 140, a humanized anti-CCR5 monoclonal antibody with potent antiviral activity in vitro (Abstract 531). Subjects receiving PRO 140 monotherapy as part of pharmacokinetics and antiviral activity studies were included in the analysis. PRO 140 was administered as either a single dose or in 3 weekly doses. All 84 subjects had R5-tropic virus at baseline as determined by the original tropism assay. Six subjects experienced a tropism shift while taking PRO 140, 4 of whom had pretreatment DM virus when baseline samples were reanalyzed using the enhanced-sensitivity tropism assay. The mechanism of tropism switch in the 2 other subjects remains under investigation. There was no statistically significant change in IC_{50} or maximal percent inhibition to PRO 140 at the end of the study and at viral rebound.

Resistance to Integrase Strand Transfer Inhibitors

Miller and colleagues examined the impact of a short course of raltegravir monotherapy on the emergence of raltegravir RAMs and the durability of subsequent raltegravir treatment (Abstract 557). The analysis was conducted in subjects participating in Protocol 004, a 2-part randomized controlled trial comparing raltegravir with efavirenz, coadministered with tenofovir/emtricitabine, in treatment-naive patients. In part 1, 35 participants received raltegravir monotherapy or placebo for 10 days as part of a raltegravir dose-ranging investigation. The authors compared subsequent raltegravir outcomes in patients who received raltegravir versus placebo in part 1 and were later treated with raltegravir in part 2. Additional inclusion criteria for this analysis were a reduction in plasma HIV-1 RNA level by 0.5 to 3 $\log_{10}$ copies/mL in part 1 and availability of a part 2 baseline genotype sample. Deep sequencing was performed and the following mutations examined: Y143C/H/R, Q148H/K/R, N155H, L74M, E92Q, T97A, E138K, G140A, V151U, and S230R. Seventeen patients were included in the analysis.

Prior to raltegravir monotherapy exposure, the RAMs Y143C, E138K, and S230R were each detected in a single subject. These mutations were seen in less than 4% of sequences obtained per subject. In 1 subject, G140S was detected in 3.04% of sequences while the subject was receiving raltegravir monotherapy. At the end of raltegravir monotherapy, 5 subjects had the following RAMs detected: Y142H, V151I, and E138K. These RAMs were seen in less than 1.5% of sequences. The detection of these RAMs had no impact on subsequent raltegravir virologic activity as measured by viral suppression at 96 weeks in part 2. The authors concluded that low levels of raltegravir RAMs, either at baseline or during raltegravir monotherapy, did not result in increased rates of virologic failure during subsequent raltegravir therapy.

Roquebert and colleagues described raltegravir-associated mutations in HIV-2-infected patients for whom a raltegravir-containing regimen was failing (Abstract 558). Seven heavily pretreated HIV-2-infected patients with incomplete viral suppression (defined as plasma HIV-2 RNA level > 100 copies/mL while receiving raltegravir and optimized background therapy) were identified through the ANRS (French National Agency for Research on AIDS and Viral Hepatitis) HIV-2 cohort. At failure, the genetic pathways associated with raltegravir resistance in HIV-2 included Y143C, Q148R/K, and N155H. In 6 of 7 samples these mutations, which mirror the primary positions for raltegravir resistance observed in HIV-1, were accompanied by the additional mutations T97A, G140S, and E92Q.

Marcelin and colleagues examined the prevalence of baseline mutations to the investigational INSTI S/GSK1349572 in INSTI-naive and raltegravir-treated patients (Abstract 554). Samples from 650 INSTI-naive patients and 84 patients experiencing raltegravir failure were sequenced for the presence of T124A, T124A/S153F,

S153Y, T124A/S153Y, and L101I, T124A/S153Y. All patients were infected with subtype-B HIV-1. INSTI-naive subjects included both antiretroviral therapy–naive and antiretroviral therapy–experienced individuals. In INSTI-naive patients, the mutations L101I and T124A were found in frequencies of 45.8% and 24.5%, respectively, and were described as polymorphisms. The S153Y/F substitutions were not observed in INSTI-naive subjects, either alone or in combination with other mutations. However, the mutations T124A and L101I/T124A were statistically significantly more frequent in subjects for whom raltegravir was failing than in INSTI-naive subjects.

Pharmacokinetic Considerations

Cervicovaginal Raltegravir Concentrations

Clavel and colleagues evaluated the concentrations of raltegravir in cervicovaginal fluid in 14 HIV-infected women receiving raltegravir-containing antiretroviral therapy (Abstract 608). They found that raltegravir concentrations were 2.3-fold higher in cervicovaginal fluids than in plasma. This confirms similar findings from HIV-uninfected women.

Effect of Etravirine on Darunavir and Raltegravir

Investigators Barrail-Tran and colleagues enrolled 12 participants from the ANRS TRIO trial in an intensive pharmacokinetic substudy to examine the interaction of etravirine with raltegravir and darunavir/r (Abstract 606). Participants in this substudy received darunavir, ritonavir, and raltegravir plus an investigator-selected background regimen of nRTIs with or without enfuvirtide. Participants added etravirine after 2 weeks. Investigators found that darunavir concentrations were increased in the presence of etravirine. Raltegravir concentrations were highly variable between participants and increased in the presence of etravirine. The observed levels of etravirine were lower than that reported in healthy volunteers. The overall safety and efficacy of this combination suggests that these interactions are not clinically important.

Nevirapine and Rifampicin

Lamorde and colleagues investigated the pharmacokinetics of nevirapine in 18 HIV-infected adults receiving rifampicin for TB (Abstract 602). Subjects were randomly assigned to start nevirapine at full dose (400 mg daily) or at lead-in dosing (200 mg once daily for 14 days followed by 400 mg daily). They found that subjects with the lead-in dosing had 40% lower exposure to nevirapine at treatment initiation. They also noted that the majority of subjects had 12-hour nevirapine concentrations that were below the minimum effective concentration. Further studies are needed on alternative dosing strategies.

Nevirapine and Antimalarial Medications

Kredo and colleagues investigated the interaction between nevirapine and lumefantrine concentrations in HIV-infected adults (Abstract 603). They enrolled 18 HIV-infected adults not receiving antiretroviral therapy and 18 stable on nevirapine-containing treatment. All participants received 6 doses of artemether/lumefantrine and underwent intensive pharmacokinetic sampling for 72 hours, with additional samples taken through 21 days. Subjects taking nevirapine had higher exposure to lumefantrine and were more likely to have therapeutic lumefantrine concentrations at day 7 (6 of 18 subjects not taking antiretroviral drugs had suboptimal concentrations compared with 1 of 18 taking nevirapine; $P = .06$). No differences were found between groups in observed adverse events or corrected QT intervals. The results were surprising, as the authors predicted that lumefantrine exposure would be decreased by nevirapine. Further study is clearly needed given the frequent clinical use of this drug combination in many parts of the world.

Atazanavir Exposure in HIV-Infected Women

Gandhi and colleagues performed intensive pharmacokinetic sampling in 122 women receiving atazanavir in the WIHS (Womens Interagency HIV Study) to identify factors associated with atazanavir exposure (Abstract 617). As expected, ritonavir coadministration resulted in a statistically significantly higher atazanavir exposure. The 24-hour area under the curve was reduced by 47% in women receiving hormonal contraception. Atazanavir concentrations were increased in those with renal insufficiency and in patients with higher bilirubin levels.

Buprenorphine/Naloxone and Once-Daily Lopinavir/Ritonavir

Bruce and colleagues examined the pharmacokinetics of buprenorphine/naloxone with and without once-daily lopinavir/r in HIV-uninfected subjects stabilized with 3 or more weeks of buprenorphine/naloxone therapy (Abstract 620). They did not find any statistically significant changes in buprenorphine/naloxone concentrations after 10 days of once-daily lopinavir/r except for increased clearance of a buprenorphine metabolite. No subjects exhibited opioid withdrawal. The authors concluded that no dose modification of buprenorphine/naloxone was needed when coadministering with lopinavir/r.

Antiretroviral Therapy and Emergency Hormonal Contraception

Carten and colleagues examined the effect of efavirenz on levonorgestrel given at doses standard for emergency contraception in 24 HIV-uninfected women (Abstract 934). Subjects were given a single dose of levonorgestrel before and after 14 days of efavirenz. The exposure to levonorgestrel was statistically significantly reduced with efavirenz. The 12-hour area under the curve and maximal concentration were reduced by 58% and 45%, respectively. The concentrations of levonorgestrel needed for efficacy are unknown, and the authors suggest that further stud-

ies should investigate alternative dosing strategies.

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