

Advances in Antiretroviral Therapy

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The 14th Conference on Retroviruses and Opportunistic Infections provided a forum for presentation of state-of-the-art research on antiretroviral therapy. This year's conference marked the first public presentation of phase III trials of the lead compounds in 2 new drug classes: maraviroc (a CCR5 inhibitor) and raltegravir (an HIV-1 integrase inhibitor). These agents are likely to be approved by the US Food and Drug Administration this year and should provide major new options for treatment-experienced patients with multidrug resistant virus. Other dominant themes of the conference were the impressive number of presentations describing outcomes of antiretroviral therapy programs in resource-limited settings and new information on mechanisms of drug resistance. Among the latter, the importance of drug resistance mutations occurring in the RNase H and connection domains of the HIV-1 reverse transcriptase was of special note. In addition, substantial new information was presented on other new antiretroviral agents, studies in treatment-naïve patients, antiretroviral therapy strategies, prevention of mother-to-child transmission, predictors of clinical response to therapy, and antiretroviral pharmacokinetics. Research in antiretroviral therapy remains dynamic and advances in the field continue to improve our ability to maintain long-term control of HIV-1 replication in infected persons.

New Antiretrovirals

A major focus of this year's conference was the presentation of phase III studies of entry and integrase inhibitors, which showed good clinical outcomes with acceptable side effect profiles. (see Table 1) These drugs are likely to be approved by the US Food and Drug Administration (FDA) in 2007 and will provide new options for treatment-experienced patients with multidrug resistant HIV-1. Studies on antiretrovirals in early-phase and preclinical development highlighted promising drugs that will hopefully further expand antiretroviral regimens available to HIV-seropositive patients.

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Antiretrovirals in Late-phase Clinical Development

Entry Inhibitors: Maraviroc. Outcomes of 2 phase IIb/III studies of maraviroc, an investigational CCR5 inhibitor, were presented: MOTIVATE 1 (Abstract 104aLB) and MOTIVATE 2 (Abstract 104bLB). The studies were of identical design but conducted in different countries. Eligible subjects were 3-class experienced patients with plasma HIV-1 RNA levels above 5000 copies/mL and exclusive use of CCR5 coreceptor for entry as determined by tropism testing. Subjects were randomized 1:2:2 to placebo, maraviroc 150 mg once daily, and maraviroc 150 mg twice daily, all given with an optimized background regimen (OBR) of 3 to 6 antiretrovirals. If subjects were not receiving a ritonavir-boosted protease inhibitor (PI), they received maraviroc 300 mg once or twice daily. Both studies had similar participant baseline characteristics.

In MOTIVATE 1, 585 subjects were enrolled (approximately 82% were white and 90% were male). Across groups the median ranges were baseline CD4+ counts of 150 to 168 cells/ μ L and base-

line HIV-1 RNA of 4.8 to 4.9 \log_{10} copies/mL. The primary endpoint, a reduction in plasma HIV-1 RNA at 24 weeks, was statistically significantly greater in the once-daily (1.82 \log_{10} copies/mL) and twice-daily arms (1.95 \log_{10} copies/mL) than in the placebo arm (1.03 \log_{10} copies/mL). Subjects in the once- and twice-daily arms were more likely to achieve plasma HIV-1 RNA levels below 50 copies/mL at week 24 than subjects in the placebo arm (42% and 49% vs 25%, respectively), and had a greater increase in CD4+ counts (107 and 111 vs 52 cells/ μ L, respectively). There were no marked safety issues and the rates of adverse events in the maraviroc arm were not statistically significantly different from rates in the placebo arm, including liver-related adverse events and malignancies.

In MOTIVATE 2, 464 subjects were enrolled (approximately 85% of subjects were white and 84% were male). Across groups the median ranges were baseline CD4+ counts of 174 to 182 cells/ μ L and baseline HIV-1 RNA of 4.8 to 4.9 \log_{10} copies/mL. The primary endpoint, reduction in plasma HIV-1 RNA at 24 weeks, was significantly greater in once-daily (1.95 \log_{10} copies/mL) and twice-daily arms (1.97 \log_{10} copies/mL) than in the placebo arm (0.93 \log_{10} copies/mL). Subjects in the once- and twice-daily arms were more likely to achieve a plasma HIV-1 RNA level below 50 copies/mL at week 24 than subjects in the placebo arm (41% and 46% vs 21%, respectively), and had a greater increase in CD4+ count (112 and 102 vs 64 cells/ μ L, respectively). There were no marked safety issues and rates of adverse events in the maraviroc arm were not statistically significantly different from rates in the placebo arm, including liver-related adverse events and malignancies.

The combined analysis from these 2 trials showed that 56% of subjects who screened for this study had HIV-1 that utilized only the CCR5 coreceptor for

Table 1. Selected Trials of Investigational Antiretroviral Drugs in Treatment-experienced Patients

Study Name Abstract No. Description	Regimen(s) (No. Patients)	Population	Baseline CD4+ cells/ μ L	Log ₁₀ Copies HIV RNA/mL
MOTIVATE-1 Abstract 104aLB Phase IIb/III randomized, double-blind trial of maraviroc, an investigational CCR5 inhibitor	Best available regimen (PIs, nRTIs, +/- enfuvirtide) (n=118) vs maraviroc 150 mg qd (n=232) or maraviroc 150 mg bid (n=235)	3-class experienced; plasma HIV-1 RNA >5000 copies/mL; CCR5-using virus	150-168 (median)	4.8-4.9 (mean)
MOTIVATE-2 Abstract 104bLB Phase IIb/III randomized, double-blind trial of maraviroc	Best available regimen (PIs, nRTIs +/- enfuvirtide) (n=91) vs maraviroc 150 mg qd (n=182) or maraviroc 150 mg bid (n=191)	1 or more major PI mutations, 3-class experience	174-182 (median)	4.8-4.9 (mean)
BENCHMRK-1 Abstract 105aLB Phase III, randomized, placebo-controlled trial of raltegravir (MK0518), an investigational integrase inhibitor	Raltegravir 400 mg bid (n=232) or placebo with OBR (n=118)	Genotypic or phenotypic resistance to at least 1 drug from all 3 current classes, plasma HIV-1 RNA >1000 copies/mL	153-156 (mean)	4.5-4.6 (mean)
BENCHMRK-2 Abstract 105bLB Phase III, randomized, placebo-controlled trial of raltegravir	Raltegravir 400 mg bid (n=230) or placebo with OBR (n=119)	Genotypic or phenotypic evidence of resistance to at least 1 drug from all 3 current classes, plasma HIV-1 RNA >1000 copies/mL	146-163 (mean)	4.5-4.6 (mean)
The HIV Integrase Inhibitor GS-9137 Demonstrates Potent ARV Activity in Treatment-experienced Patients Abstract 143LB Phase II, dose-finding study of elvitegravir (GS9137), an investigational integrase inhibitor	Elvitegravir (20 mg, 50 mg, or 125 mg) + ritonavir 100 mg qd or best available control PI given with OBR (n=278)	1 or more PI mutations, plasma HIV-1 RNA >1000 copies/mL	157-243 (median)	4.5-4.7 (median)

PI indicates protease inhibitor; nRTI, nucleoside analogue reverse transcriptase inhibitor; OBR, optimized background regimen; qd, once-daily; bid, twice daily.

entry (R5 HIV). Eight percent of subjects who had R5 HIV at screening had dual or mixed HIV-1 populations that utilized both CCR5 and CXCR4 coreceptors for entry at baseline, prior to receiving maraviroc. The dual or mixed HIV subjects who received maraviroc had a poorer virologic response than R5 HIV subjects who received maraviroc. These results are similar to what was observed in a previously presented trial of maraviroc in dual or mixed HIV subjects. The number of ac-

tive drugs in the OBR was found to be a predictor of suppression to less than 50 HIV-1 RNA copies/mL and baseline HIV-1 RNA viral load was not related. The proportion of patients achieving HIV-1 RNA below 50 copies/mL in the once- and twice-daily arms were 18% and 29%, respectively, among patients with no active drugs in the OBR; 43% and 43% with 1 active drug in the OBR; 52% and 53% with 2 active drugs in the OBR; and 61% and 58% with 3 or more active drugs

in the OBR, indicating that difference in viral load outcomes between once- and twice-daily maraviroc was apparent only among patients with no active drugs in the OBR.

Among patients with virologic failure, change in coreceptor usage from R5 HIV to dual or mixed HIV was noted in 4 of 84 (5%) subjects in the placebo groups, 31 of 49 (63%) subjects in the once-daily groups, and 32 of 49 (65%) of subjects in the twice-daily groups.

Follow-up Time	HIV-1 RNA Response	Comments
24 weeks	-1.03 vs -1.82 and -1.95 log ₁₀ copies/mL 25% vs 42% and 49% <50 copies/mL	Combined analyses: The qd and bid dosing groups appeared no different when having 1 or more active drugs in OBR; in 31/49 subjects in whom maraviroc failed, there was a change in coreceptor usage
24 weeks	-0.93 vs -1.95 and -1.97 log ₁₀ copies/mL 21% vs 46% and 41% <50 copies/mL	
16 weeks	77% vs 41% <400 copies/mL 61% vs 33% <50 copies/mL	Combined analyses: 32/41 subjects in whom raltegravir failed had mutations in integrase; 98% of subjects receiving raltegravir and enfuvirtide for the first time had <400 HIV-1 RNA copies/mL at week 16
16 weeks	77% vs 43% <400 copies/mL 62% vs 36% <50 copies/mL	
16 weeks	50-mg arm, -1.5 log ₁₀ copies/mL 125-mg arm, -1.7 log ₁₀ copies/mL vs control arm, -1.2 log ₁₀ copies/mL	Elvitegravir led to rapid declines in plasma HIV-1 RNA at week 2 that were sustained only if there were other active drugs in the OBR 20-mg arm stopped early for virologic failure

the median ranges were baseline CD4+ counts of 153 to 156 cells/μL and baseline HIV-1 RNA of 4.5 to 4.6 log₁₀ copies/mL. The OBR contained 0 or 1 active drug in 48% to 51% of subjects; 20% to 21% received enfuvirtide for the first time and 25% to 27% received darunavir for the first time. More subjects in the raltegravir group achieved plasma HIV-1 RNA levels below 400 copies/mL (77%) and below 50 copies/mL (61%) at week 16 than subjects in the placebo group (41% and 33%, respectively). Subjects receiving raltegravir had a greater increase in CD4+ count than those in the placebo group (83 cells/μL vs 31 cells/μL).

Similar results were observed in BENCHMRK-2 (Abstract 105bLB). Of the 349 subjects enrolled, approximately 60% were white and 90% were male. Across groups the median ranges were baseline CD4+ counts of 146 to 163 cells/μL and baseline HIV-1 RNA of 4.5 to 4.6 log₁₀ copies/mL. The OBR contained 0 or 1 active drug in 44% to 46% of subjects. Nineteen percent to 20% received enfuvirtide for the first time and 45% to 50% received darunavir for the first time. More subjects in the raltegravir group achieved plasma HIV-1 RNA levels below 400 copies/mL (77%) and below 50 copies/mL (62%) at week 16 than did subjects in the placebo group (43% and 32%, respectively). Subjects receiving raltegravir had a greater increase in CD4+ count than those in the placebo group (86 cells/μL vs 40 cells/μL).

The authors presented data on combined analysis of both studies. Genotypic resistance data were available for 41 subjects who had virologic failure while receiving raltegravir. Thirty-two of 41 had resistance-associated mutations in integrase, and mutations fell generally into one of 2 mutational pathways: N155H or Q148K/R/H. One of these mutations was usually present with additional integrase mutations. These pathways were expected based on in vitro data. Subgroup analysis showed that 98% of subjects receiving raltegravir with de novo use of both enfuvirtide and darunavir achieved HIV-1 RNA levels below 400 copies/mL at 16 weeks,

There were no obvious adverse effects that resulted from change in coreceptor usage. CD4+ cell counts were generally well preserved in subjects who experienced change in coreceptor usage.

Integrase Inhibitors: Raltegravir and GS-9137. Investigators presented data from 2 phase III studies of the investigational HIV-1 integrase inhibitor raltegravir (MK-0518), BENCHMRK-1 and BENCHMRK-2 (Abstracts 105aLB,

105bLB). The 2 studies were identical in design. Inclusion criteria included evidence of genotypic or phenotypic resistance to at least 1 drug from each of 3 classes and plasma HIV-1 RNA levels of above 1000 copies/mL. Subjects were randomized 2:1 to raltegravir 400 mg twice daily or placebo in each study.

In BENCHMRK-1 (Abstract 105aLB), 350 subjects were enrolled (approximately 78% of subjects were white and 85% were male). Across groups

and 90% of subjects receiving raltegravir with de novo use of either enfuvirtide or darunavir achieved HIV-1 RNA levels below 400 copies/mL at week 16. The overall rate of serious drug-related adverse events was low in both studies and no specific adverse events were associated with raltegravir use.

Zolopa and colleagues (Abstract 143LB) presented data from a phase II dose-finding study of the investigational integrase inhibitor GS-9137. Eligible subjects had plasma HIV-1 RNA levels above 1000 copies/mL and 1 or more PI mutations. There were 278 subjects randomized to 1 of 3 doses of GS-9137 (20 mg, 50 mg, or 125 mg with 100 mg ritonavir once daily) or best available PI. The subjects were 90% male and 73% white, and 23% used enfuvirtide for the first time. The median baseline CD4+ count was 157 to 243 cells/ μ L and mean baseline HIV-1 RNA level was 4.5 to 4.7 \log_{10} copies/mL. The 20-mg arm was stopped early due to inferior virologic performance. Through 16 weeks, the control arm had an average virologic reduction of 1.2 \log_{10} HIV-1 RNA copies/mL compared with 1.5 \log_{10} copies/mL in the 50-mg group and 1.7 \log_{10} copies/mL in the 125-mg group. Although this was a noninferiority study design, the 125-mg group had a statistically significantly greater viral load reduction than the control group. Thirty-eight percent and 40% of subjects receiving the 2 higher doses achieved below 50 HIV-1 RNA copies/mL at week 16 compared with 30% of subjects in the control arm. Subjects with no active drugs in the OBR tended to have a rapid drop in plasma HIV-1 RNA at week 2 followed by virologic rebound, whereas subjects with 1 or more active drugs in the OBR had more sustained virologic suppression. Thus, GS-9137 was well tolerated and no marked safety concerns were identified. This study highlights the importance of supporting integrase inhibitor use with other active drugs in the regimen to avoid the rapid emergence of drug resistance to this new class.

New Reverse Transcriptase Inhibitors: Rilpivirine (TMC278) and (\pm)- β -2', 3'-dideoxy-3'-thia-5-fluorocytosine

(\pm)-FTC Pozniak and colleagues (Abstract 144LB) presented data from the TMC278-C204 trial, a phase IIb study of an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI) with retained antiviral activity against HIV and resistant to currently FDA-approved NNRTIs, in treatment-naive subjects. Subjects were randomized to 1 of 3 doses of rilpivirine or efavirenz once daily, given with fixed-dose tenofovir/emtricitabine or zidovudine/lamivudine. The median baseline plasma HIV-1 RNA was 4.9 \log_{10} copies/mL and the median CD4+ count was 203 cells/ μ L. Three hundred sixty-eight subjects (33% women and 53%-56% nonwhite) were randomized. In an intention-to-treat analysis, 77% to 81% of subjects in the rilpivirine arms achieved the primary endpoint of below 50 HIV-1 RNA copies/mL compared with 81% in the efavirenz arm, a difference that was not statistically significant. Increases in CD4+ count were similar in both arms (123-145 cells/ μ L in the rilpivirine arms vs 125 cells/ μ L in the efavirenz arm; P = not significant). There were, however, better overall lipid profiles and significantly lower rates of rash and central nervous (CNS) system side effects in the rilpivirine arms than in the efavirenz arm. The rates of serious adverse events were low and there was not a statistically significant difference between groups. Complete resistance analysis is underway and was not available at this presentation.

(\pm)-FTC is an investigational mixture of emtricitabine plus its D-enantiomer. Cahn and colleagues (Abstract 488) presented data on a double-blind, phase II trial of (\pm)-FTC in treatment-experienced subjects. Entry criteria included current virologic failure on a lamivudine-containing regimen and presence of the M184V mutation. Subjects were randomized to receive (\pm)-FTC 600 mg and discontinue lamivudine (n = 26) or continue lamivudine (n = 16) for 28 days. The lamivudine group had an increase in plasma HIV RNA-1 of 0.13 \log_{10} copies/mL compared with a decrease of 0.4 \log_{10} copies/mL in the (\pm)-FTC group (P < .01). Subgroup analysis showed that sub-

jects with less than 3 thymidine analogue mutations (TAMs) accounted for the majority of the benefit (reduction of 0.7 \log_{10} copies/mL). No obvious safety issues were noted.

Antiretrovirals in Phase I and Preclinical Development

Nucleoside Reverse Transcriptase Inhibitors: IDX12899 and IDX12989. Richman and colleagues (Abstract 489) presented data on 2 investigational NNRTIs: IDX12899 and IDX12989. Both compounds exhibited potent activity against a broad range of clinical isolates including isolates with single- and double-NNRTI mutations. The pharmacokinetic profiles in various animal models supported once-daily dosing. No adverse events were noted in acute toxicology models. Serial passage studies exhibited a longer time for development of resistance to these compounds than to efavirenz.

Protease Inhibitors: GS-8374. Callebaut and colleagues (Abstract 491) presented data on a novel peptidomimetic PI, GS-8374. In vitro studies showed potent activity against a range of clinical isolates including those with resistance to darunavir and brexnavir. Development of resistance to this compound did not occur after 6 months of serial passage experiments despite development of resistance to lopinavir, atazanavir, and darunavir in parallel experiments. GS-8374 showed minimal effects on lipid accumulation and insulin-stimulated glucose uptake in adipocytes.

Integrase Inhibitors: MK-2048 and GSK364735. Wai and colleagues (Abstract 87) presented data on a new series of compounds that inhibit the strand transfer reaction of HIV integrase and are similar to 2 integrase inhibitors in late-stage clinical development (naphthyridine [L-870810] and pyrimidinone [MK-0518, raltegravir]), but were designed to have a higher genetic barrier to resistance and limited cross-resistance to previously described compounds. One compound, MK-2048, was found to have a 95% inhibitory concentration (IC_{95}) of 41 nM

and a pharmacokinetic profile in dogs and rats that suggests once-daily dosing in humans is possible, and demonstrated retained activity against HIV-1 strains resistant to integrase inhibitors currently in clinical development.

Reddy and colleagues (Abstract 562) presented data on the safety and pharmacokinetics of an HIV-1 integrase inhibitor, GSK-364735, in HIV-uninfected subjects. Among 79 subjects, they found only mild adverse events except for 1 moderate headache. No grade 2 or higher laboratory events were noted. Food increased the bioavailability of this compound by 30% to 100%, and aluminum and magnesium hydroxide decreased bioavailability by 50%. The compound did not markedly alter CYP450 enzymes except for weak inhibition of CYP1A2 and the pharmacokinetic profile was not affected by ritonavir. The pharmacokinetic data supported twice-daily dosing in future phase II studies.

CXCR4 Inhibitors: AMD11070. Two studies evaluated AMD11070, an investigational oral CXCR4 inhibitor: the X4 Antagonist Concept Trial (XACT), presented by Moyle and colleagues (Abstract 511), and AIDS Clinical Trials Group (ACTG) A5210 presented by Saag and colleagues (Abstract 512). Both were phase I studies that evaluated exposure to 10 days of AMD11070 given twice daily. Eligible subjects were off antiretroviral therapy, had plasma HIV-1 RNA levels above 5000 copies/mL, and above 2000 relative luminescence units (RLU) of CXCR4 usage in the trofile assay. Nearly all subjects received 200 mg of AMD11070 twice daily for 10 days. Four of 9 in XACT and 3 of 6 in ACTG 5210 subjects had at least a 1- \log_{10} reduction in X4 RLU (indicating a decrease of plasma HIV-1 using CXCR4 for entry in CD4+ cells) after 10 days of monotherapy. Both studies concluded that proof-of-concept has been established and that further studies are warranted. The clinical development of AMD11070 has been put on hold due to abnormal liver histology in long-term toxicology studies in animals.

Morpholino Antisense Oligonucleotides. Phosphorodiamidate morpholino oligomers (PMOs) are water-soluble

antisense oligonucleotide analogues that block complementary RNA sequences. Bestwick and colleagues (Abstract 499) investigated the potential for PMO to act against the highly conserved start codon region of the HIV-1 *vif* gene and the Tar stem-loop. They found that the optimal *vif* PMO generated was able to inhibit viral replication with a median effective concentration (EC_{50}) of 260 nM whereas the Tar stem-loop PMO inhibited with an EC_{50} of 3.6 μ M. These data support pursuing this strategy for antiretroviral drug development.

Histone Deacetylase Inhibitors. Latently infected, resting memory CD4+ T cells are a major reservoir for HIV and represent a significant barrier to eradication of HIV from infected individuals. Histone deacetylases (HDACs), of which there are 3 classes (I, II, and III), are important in maintaining viral latency by causing the DNA to become tightly bound, thus preventing access to HIV DNA by nuclear transcription factors. HDAC inhibitors may lead to expression of HIV genes resulting in productive infection, which would then expose the previously latent HIV-infected cells to immune surveillance and the effects of antiretroviral drugs. Valproic acid is a known nonspecific HDAC inhibitor. Weiman and colleagues (Abstract 500) presented data on several small molecules that inhibit class I HDACs and showed that these molecules promoted histone acetylation. Archin and colleagues (Abstract 501) then tested these HDAC inhibitors on latently infected CD4+ cells and showed that they increased viral transcription and effectively withdrew cells from latency.

Vpu Ion Channels. Vpu has been shown to have 2 important functions in the lifecycle of HIV-1: virion assembly and release, and CD4+ degradation. It associates in pentamers to form a cation-specific ion channel. Luscombe and colleagues (Abstract 502) presented data on several inhibitors of the Vpu ion channel. Such inhibitors should be effective at interfering with viral replication in macrophages, a reservoir not targeted by currently available drugs. The lead compound, BIT225, showed potent an-

tiretroviral activity at an EC_{50} 1.1 μ M in HIV-1-infected macrophages. The 50% cytotoxicity concentration was 212 μ M, suggesting a favorable antiviral index. It showed broad activity against a range of clinical isolates and appeared to be synergistic with several antiretrovirals.

Clinical Trials in Treatment-naive Patients

Trials in Treatment-naive Patients with Established HIV-1 Infection

Results of selected treatment trials in antiretroviral-naive patients are summarized in Table 2.

Mildvan and colleagues (Abstract 138) presented results of the A5073 trial, a 48-week, multicenter, 3-arm, open-label trial that compared lopinavir 400 mg/ritonavir 100 mg soft gel capsule self-administered twice daily, lopinavir 800 mg/ritonavir 200 mg self-administered once daily, and lopinavir 800 mg/ritonavir 200 mg via directly observed therapy once daily. Participants were antiretroviral-naive with HIV-1 RNA levels above 3.3 \log_{10} copies/mL and were randomized in a 2:2:1 ratio stratified by screening HIV-1 RNA levels above or below 5.0 \log_{10} copies/mL. All patients received emtricitabine 200 mg with extended-release stavudine 100 mg or tenofovir 300 mg once daily. The 402 patients enrolled had a baseline median CD4+ count of 197 cells/ μ L and median HIV-1 RNA level of 4.8 \log_{10} copies/mL. The difference in plasma HIV-1 RNA at 48 weeks between the once- and twice-daily groups was 0.03 \log_{10} copies/mL (95% confidence interval [CI], -0.07-0.12). However, in the higher HIV-1 RNA level stratum, the probability of sustained virologic response at 48 weeks was statistically significantly higher in the twice-daily group than in the once-daily self-administered group (0.89; 95% CI, 0.79-0.94 vs 0.76; 95% CI, 0.64-0.84, respectively). Probabilities of sustained virologic response at 24 and 48 weeks between the directly observed therapy and once-daily self-administered arms were not statistically significantly different.

Rey and colleagues (Abstract 503)

presented the preliminary results of the DAUFIN study, a randomized, open-label, multicenter, noninferiority trial of once-daily lamivudine 300 mg, tenofovir 245 mg, and nevirapine 400 mg versus twice-daily zidovudine 300 mg/lamivudine 150 mg and nevirapine 200 mg. The trial was stopped by the steering committee after 12-week data showed 7 early nonresponses (defined as plasma HIV-1 RNA with a less than $2.0 \log_{10}$ copies/mL decrease or rebound of more than $1.0 \log_{10}$ copies/mL after initial decrease) in the once-daily arm and no early nonresponses in the twice-daily arm. The early nonresponders had higher baseline median plasma HIV-1 RNA levels and lower median CD4+ count, and all had 1 or more NNRTI mutations. Viral genotypes showed that 6 of the 9 individuals who did not respond to therapy in the once-daily arm also had K65R mutations.

Walker and colleagues (Abstract 506) presented 48-week results of the Evaluating the Safety of Nevirapine or Abacavir (NORA) trial, a randomized substudy of the Development of Antiretroviral Therapy in Africa (DART) trial in Uganda of 600 antiretroviral-naive patients with CD4+ counts below 200 cells/ μ L. The trial compared zidovudine 300 mg/lamivudine 150 mg plus abacavir 300 mg twice daily with zidovudine 300 mg/lamivudine 150 mg plus nevirapine 200 mg twice daily and was placebo-controlled for the first 24 weeks. Baseline median CD4+ count was 99 cells/ μ L and mean plasma HIV-1 RNA was $5.4 \log_{10}$ copies/mL. At 48 weeks, 77% of the nevirapine arm and 62% of the abacavir arm had plasma HIV-1 RNA levels below $1.7 \log_{10}$ copies/mL ($P < .001$). The CD4+ cell count increase at 48 weeks was statistically significantly higher in the nevirapine arm (173 cells/ μ L) than in the abacavir arm (147 cells/ μ L; $P = .006$). Despite the greater HIV-1 RNA level decreases and CD4+ cell increases, the authors noted a trend suggesting clinical outcome superiority of the abacavir arm at 48 weeks, with 29 (10%) of individuals in the nevirapine arm developing new World Health Organization (WHO) stage IV events or death,

compared with 17 (6%) in the abacavir arm ($P = .06$). A similar trend was observed for WHO stage III events, but was not statistically significant.

Bussmann and colleagues (Abstract 507) presented preliminary results from the Tshepo Study, an open-label, randomized, ongoing study in Botswana examining 3 different nucleoside reverse transcriptase inhibitor (nRTI) regimens, 2 NNRTI regimens, and community-based directly observed therapy versus standard of care. The study enrolled 650 antiretroviral-naive patients between December 2002 and December 2004. Sixty-nine percent of the patients were female and baseline patient age, CD4+ count, and median follow up were 35.9 years, 199 cells/ μ L, and 89.7 weeks, respectively. The patients were randomized in a $3 \times 2 \times 2$ factorial design to: A) zidovudine/lamivudine, zidovudine/didanosine, or stavudine/lamivudine; B) efavirenz or nevirapine; and C) community-based directly observed therapy or standard of care. There were also 2 balanced strata of CD4+ cell counts: below 201 cells/ μ L with any viral load, and between 201 and 350 cells/ μ L with plasma HIV-1 RNA above $4.74 \log_{10}$ copies/mL. The analysis was undertaken after the Data and Safety Monitoring Board (DSMB) suspended the zidovudine/didanosine-containing arm owing to inferiority in the primary endpoint of virologic failure. The authors pooled data from the 2 NNRTI arms and compared patients receiving zidovudine/didanosine with those receiving zidovudine/lamivudine or stavudine/lamivudine. The rate of virologic failure with genotypic resistance was 11% in the zidovudine/didanosine arm and 2% in the zidovudine/lamivudine or stavudine/lamivudine arm ($P = .0002$). No difference in death rate or time to first treatment-limiting toxicity was observed in either nRTI arm. Unexpectedly, there were no differences between the community-based directly observed therapy and standard-of-care arms, which the authors suggested was due to overall low rates of virologic failure. No results were given comparing efavirenz and nevirapine groups.

Trials in Treatment-naive Patients with Acute HIV-1 Infection

Estes and colleagues (Abstract 67) examined the impact of antiretroviral treatment on CD4+ cell populations in lymph nodes, Peyer's patches, and the lamina propria by obtaining inguinal lymph node and ileum biopsies in 32 HIV-1-infected and 11 HIV-seronegative individuals. They obtained follow-up biopsies in 15 of the HIV-1-infected individuals 6 months after initiation of potent antiretroviral therapy. All compartments were significantly depleted of CD4+ cells in HIV-1-infected patients and, after initiation of antiretroviral therapy, there was an increase in CD4+ cells in peripheral blood and lymph nodes, but not in the lamina propria. In patients who began antiretroviral therapy in the acute and early stages of infection, the mean increase in peripheral blood CD4+ counts was 388 cells/ μ L and the parafollicular T-cell zone was 12.5% occupied. In patients who initiated therapy with established infection in the presymptomatic phase the increase in peripheral blood CD4+ count was 176 cells/ μ L and the parafollicular T-cell zone was 13.65% occupied. Earlier initiation of antiretroviral therapy was associated with greater increases in the central memory cell population of the Peyer's patches. There was no change in measured T-cell subsets in patients who initiated antiretroviral therapy after being diagnosed with AIDS.

Thus far, studies of the immunologic and virologic outcomes of early treatment of primary HIV disease have been contradictory.¹⁻³ Four abstracts at this year's conference used data from large cohort studies to examine this, but did not end controversy. Similar definitions of primary infection were used in each of the following studies.

Steingrover and colleagues (Abstract 124LB) used data from 2 large cohorts of HIV-seropositive patients in Dutch treatment centers, the Amsterdam Cohort Study and the Athena cohort, to examine the immunologic and virologic outcomes of initiating potent antiretroviral therapy within 6 months of HIV seroconversion. Of the 332 patients identified with primary HIV-1 infection, 64 were treated with potent antiretrovi-

Table 2. Selected Trials of Antiretroviral Therapy in Treatment-naive Patients

Study Name Abstract No. Description	Population	Regimen(s)	Baseline CD4+ cells/ μ L, Log_{10} copies of HIV RNA/mL	Follow-up Time	Response	Comments
TMC278-C204 Abstract 144LB Efficacy and safety testing of 3 doses of rilpivirine (TMC278), an investigational NNRTI with activity against HIV-1 resistant to currently available NNRTIs	33% female 53%-56% nonwhite	Randomized to: rilpivirine 25 mg qd rilpivirine 75 mg qd rilpivirine 150 mg qd or efavirenz 600 mg qd plus: zidovudine/ lamivudine (76%) or tenofovir/ emtricitabine (24%) (n=368)	203 (median) 4.9 (median)	48 weeks	Primary endpoint of intent-to-treat analysis: Rilpivirine arms: 77%-81% with <50 copies/mL Efavirenz arm: 81% with <50 copies/mL $P = ns$ CD4+ count rise: Rilpivirine arms: 123-145 cells/ μ L Efavirenz arm: 125 cells/ μ L $P = ns$	Some differences in side-effect profiles: Incidence of rash and central nervous system side effects were significantly lower in the rilpivirine arms. Rilpivirine associated with lower total cholesterol, lower LDL, and lower triglycerides, although the efavirenz group had a higher HDL. Complete resistance analysis was not available.
ACTG A5073 Abstract 138 Comparison of qd (with or without DOT) versus bid lopinavir/ritonavir	Plasma HIV-1 RNA >3.3 log_{10} copies/mL Stratified by plasma HIV-1 RNA < or \geq 5.0 log_{10} copies/mL Non-blinded	Lopinavir 400 mg/ ritonavir 100 mg soft gel capsule 400/100 mg, self-administered bid Lopinavir 800 mg/ ritonavir 200 mg, self-administered qd Lopinavir 800 mg/ ritonavir 200 mg via DOT qd plus: emtricitabine/ stavudine or emtricitabine/ tenofovir (n=402)	197 (median) 4.8 (median)	48 weeks	Difference in plasma HIV-1 RNA at 48 weeks between qd and bid groups was 0.03 log_{10} copies/mL (95% CI, -0.07-0.12) No significant difference in probability of sustained virologic response between DOT and qd, self-administered arms at 24 and 48 weeks	Probability of sustained virologic response at 48 weeks in higher stratum was higher in bid group (0.89; 95% CI, 0.79-0.94) than in the qd self-administered group (0.76; 95% CI, 0.64-0.84).
DAUFIN Study Abstract 503 Non-inferiority trial of qd nevirapine vs bid nevirapine Preliminary results	CD4+ count <350 cells/ μ L Non-blinded (n=71)	Lamivudine 300 mg/ tenofovir 245 mg/ nevirapine 400 mg qd (n=36) Zidovudine 300 mg/ lamivudine 150 mg/ nevirapine 200 mg bid (n=35)	207 (median) 4.85 (median) 209 (median) 4.94 (median)	12 weeks 12 weeks	7 early non-responses 19% early non-response rate 0 early non-responses	The trial steering committee stopped the study. The definition of early non-response was plasma HIV-1 RNA with either <2.0 log_{10} copies/mL decrease or rebound of >1.0 log_{10} copies/mL 1 or more NNRTI resistance mutations seen in all cases.

Table 2. Selected Trials of Antiretroviral Therapy in Treatment-naive Patients (cont'd)

Study Name Abstract No. Description	Population	Regimen(s)	Baseline CD4+ cells/ μ L, Log_{10} copies of HIV RNA/mL	Follow-up Time	Response	Comments
NORA Trial, nested substudy within DART Abstract 506 Comparison of abacavir-based vs nevirapine-based regimens	Ugandan adults 72% women median age, 36 19% WHO stage IV CD4+ count <200 cells/ μ L (n=600)	Zidovudine 300 mg/ lamivudine 150 mg, abacavir 300 mg bid Zidovudine 300 mg/ lamivudine 150mg, nevirapine 200 mg bid	99 (median) 5.4 (mean)	48 weeks	HIV-1 RNA <50 copies/mL at week 48: 62% Mean CD4+ count increase from baseline: 147 cells/ μ L HIV-1 RNA <50 copies/mL at week 48: 77% ($P < .001$) Mean CD4+ count increase from baseline: 173 cells/ μ L ($P = .006$)	More subjects in the nevirapine arm died or developed WHO grade IV events: 17 (6%) vs 29 (10%) $P = .06$
Tshepo Study Abstract 507 Comparison of didanosine- containing regimen to other nRTIs Trial also looking at 2 NNRTIs and DOT vs standard of care	Botswanan adults 69.4% female median age, 35.9 Stratified to CD4+ count <201 or 201- 350 cells/ μ L with plasma HIV-1 RNA > 4.74 log_{10} copies/mL (n=650)	Zidovudine/ didanosine + NNRTI Zidovudine/lamivudine or stavudine/lamivudine + NNRTI	199 (median) 5.3 (median)	89.7 weeks (median)	Rates of virologic failure with genotypic resistance: 11% 2% ($P = .0002$)	Increase in CD4+ cells, percentage of patients with undetectable plasma HIV-1 RNA, death rates, and time to first treatment- modifying toxicity were equivalent in the 2 arms. Zidovudine/ didanosine- containing arms were suspended by the DSMB.

NNRTI indicates nonnucleoside analogue reverse transcriptase inhibitor; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ACTG, AIDS Clinical Trials Group; CI, confidence interval; DOT, directly observed therapy; WHO, World Health Organization; nRTI nucleoside analogue reverse transcriptase inhibitor; DSMB, Data Safety Monitoring Board; qd, once-daily; bid, twice-daily.

ral therapy within 180 days of seroconversion, and 32 subsequently stopped the therapy. Higher plasma HIV-1 RNA levels at seroconversion independently predicted initiation of early antiretroviral therapy. Viral load at 7 weeks post-treatment interruption was lower in the treatment interruption group than at 7 weeks post-seroconversion among patients who did not initiate treatment (-0.6 log_{10} copies/mL; $P < .001$). This difference decreased over time, and at approximately 100 weeks the plasma HIV-1 RNA levels were indistinguishable

between the 2 groups. There was no difference in the CD4+ count decline between the untreated patients and patients after treatment interruption.

Koegl and colleagues (Abstract 125LB) performed a similar analysis using data from 2 German cohorts of patients with primary HIV-1 infection who were or were not treated with early antiretroviral therapy (the Prime-DAG cohort and the Ac-DAG cohort, respectively) and found differences in both viral load and CD4+ count in treated versus untreated groups. Of the 200 cases of primary

HIV-1 infection, 95.5% were men and 144 started antiretroviral therapy either before or during seroconversion. The median CD4+ cell count was lower and the median plasma HIV-1 RNA level was higher in the treatment group than in the non-treatment group: 453 versus 629 cells/ μ L ($P = .001$), and above 5.7 versus 5.38 log_{10} copies/mL ($P < .001$), respectively. One hundred of the 144 patients who initiated treatment stopped antiretroviral therapy after a median time of 9.5 months (range, 2.1-28.7 months). Although the plasma HIV-1 RNA levels

differed significantly between treated and untreated patients at 6 months (4.4 \log_{10} copies/mL vs 5.0 \log_{10} copies/mL; $P = .01$), they did not differ significantly at 12 months (4.58 vs 4.72 \log_{10} copies/mL; $P = \text{ns}$). The absolute CD4+ counts were not significantly different at 6 or 12 months. However, 12 months after treatment cessation, the CD4+ count had increased by 60 cells/ μL in the treated group, and 12 months after seroconversion, the CD4+ count had decreased by 86 cells/ μL in the untreated group, a statistically significant difference ($P = .01$).

Seng and colleagues (Abstract 347) found no difference in CD4+ count trends in patients in the Agence Nationale de Recherches Sur le Sida (ANRS) PRIMO cohort with primary HIV-1 infection after interruption of potent antiretroviral therapy compared with patients in the ANRS SEROCO cohort with primary HIV-1 infection who never received antiretroviral therapy. The 170 patients included from the ANRS PRIMO cohort began antiretroviral therapy within 3 months of their HIV-1 diagnosis. All responded with plasma HIV-1 RNA levels below 2.7 \log_{10} copies/mL within 6 months, continued treatment for at least 6 months (average duration, 19 months), and discontinued treatment for at least 3 months (average duration, 21 months). Mean CD4+ count at 36 months after treatment interruption in the PRIMO cohort ($n = 170$) and after HIV-1 infection in the SEROCO cohort ($n = 123$) were equal at 416 cells/ μL (95% CI for PRIMO, 369-464; for SEROCO, 360-476). Transient antiretroviral therapy initiated during primary HIV-1 infection did not extend time spent off treatment or time to a CD4+ count of below 350 cells/ μL .

In a case-controlled study of patients with primary HIV-1 infection who received potent antiretroviral therapy (Abstract 348), early antiretroviral treatment correlated with immunologic but not virologic outcomes. Eighty-nine patients with primary HIV-1 infection who received 3 months of antiretroviral therapy in the St. Mary's cohort were matched for age, sex, HIV-1 risk group, year of estimated seroconversion, and seroconversion window interval, with 179 patients from the Concerted Action on Seroconversion to AIDS and Death in

Europe (CASCADE) cohort. After adjusting for confounders, rate of CD4+ cell decline over the 3 years following seroconversion was greater in the untreated controls than in the treated case patients, with a mean decrease of 51 cells/ μL per year in the case patients (95% CI; 32-69) and 77 cells/ μL per year in the controls (95% CI; 65-89). The estimated hazard ratio (HR) for the combined events of antiretroviral treatment initiation and a CD4+ count below 350 cells/ μL was 1.445 (95% CI, 1.020-2.054, $P = .039$) for the untreated controls compared with the treated cases. Plasma HIV-1 RNA levels did not show a statistically significant difference between the 2 groups either at 18 months or 2 years after seroconversion. It is unclear what effect, if any, the significantly longer follow-up time for the case patient group (2.45 years) compared with the controls (1.20 years, $P < .001$) had on this analysis.

The above studies indicate that early antiretroviral treatment in primary HIV-1 infection may have beneficial virologic and immunologic effects, though the results are inconsistent and limited by the studies' observational natures. The question of whether early antiretroviral treatment in primary HIV-1 infection should become standard-of-care awaits results from randomized, controlled trials that are currently underway.

Antiretroviral Treatment Strategies

Lopinavir/Ritonavir Monotherapy as a De-escalation Strategy

In the OK and OK04 studies (Abstracts 513 and 638) patients on a regimen of lopinavir/ritonavir plus 2 nRTIs with plasma HIV-1 RNA levels below 1.7 \log_{10} copies/mL for at least 6 months and no history of virologic failure on PIs were randomized to either continue treatment with lopinavir/ritonavir plus 2 nRTIs or to lopinavir/ritonavir monotherapy. Data from 121 participants in both open-label trials assigned to lopinavir/ritonavir monotherapy were analyzed to identify risk factors for loss of virologic suppression, defined as a plasma HIV-1 RNA level above 1.7 \log_{10} copies/mL at 48 weeks (Abstract 513). Baseline CD4+ counts

for the patients from the OK and OK04 trials were 662 cells/ μL and 474 cells/ μL , respectively, and baseline plasma HIV-1 RNA levels before receiving antiretrovirals were 5.1 \log_{10} copies/mL in both trials. Of 121 patients, 15 had loss of virologic suppression by week 48, with a Kaplan-Meier probability of loss of virologic suppression of 12.7%. Independent factors associated with loss of virologic suppression were low adherence (HR, 6.3; 95% CI, 2.0-19.6; $P = .002$), lower baseline hemoglobin (HR per g/dL, 0.68; 95% CI, 0.50-0.92; $P = .013$) and nadir CD4+ count below 100 cells/ μL (HR, 4.1; 95% CI, 1.3-13.5; $P = .02$).

Arribas and colleagues (Abstract 638) reported drug resistance outcomes from the OK04 trial. During the trial, genotype testing was performed on all plasma samples in which HIV-1 RNA was greater than 2.7 \log_{10} copies/mL. Eleven subjects in the monotherapy arm and 4 subjects in the control arm qualified for genotype by these criteria ($P = .07$), and 2 isolates in the monotherapy arm and 1 in the control arm had major PI mutations as defined by the International AIDS Society-USA 2006 guidelines (V82A or M46I).⁴ The authors concluded that the incidence of PI resistance over the first 48 weeks of the OK04 study was low, and similar between the 2 treatment arms. They also noted that 5 of 11 patients with plasma HIV-1 RNA levels above 2.7 \log_{10} copies/mL did not have resistance mutations, remained on monotherapy, and had re-suppressed their plasma HIV-1 RNA to less than 1.7 \log_{10} copies/mL.

Campo and colleagues (Abstract 514) reported results from the M03-613 trial, which randomized 155 antiretroviral-naive patients with HIV-1 infection in a 2:1 ratio to: lopinavir/ritonavir plus zidovudine/lamivudine induction therapy for at least 24 weeks, followed by maintenance with lopinavir/ritonavir monotherapy after 3 consecutive months of plasma HIV-1 RNA levels below 1.7 \log_{10} copies/mL, or efavirenz plus zidovudine/lamivudine. Eligible subjects had plasma HIV-1 RNA levels above 1000 copies/mL, were antiretroviral naive, and had no evidence of resistance to study drugs. At 96 weeks, 60% of the lopinavir/ritonavir mono-

therapy arm and 63% of the efavirenz plus zidovudine/lamivudine arm had plasma HIV-1 RNA levels below $1.7 \log_{10}$ copies/mL. In subjects who deintensified to monotherapy, 32 had confirmed plasma HIV-1 RNA levels above $1.7 \log_{10}$ copies/mL. Time to loss of virologic response to a level above $1.7 \log_{10}$ HIV-1 RNA copies/mL was statistically significantly different between the lopinavir/ritonavir (approximately 60% maintaining response at 64 weeks) and efavirenz arms (approximately 90% maintaining response at 64 weeks; $P < .0001$). When the virologic threshold for time to loss of virologic response was raised to greater than $2.7 \log_{10}$ HIV-1 RNA copies/mL, there was no statistically significant difference between the groups. Low adherence, defined as a subject reporting at least 1 missed lopinavir/ritonavir dose ($P = .07$), and baseline CD4+ cell count ($P = .06$) predicted virologic rebound in the monotherapy group.

Treatment Interruptions

In contrast to last year's conference, few new antiretroviral therapy outcome data were presented on treatment interruption strategies. Some further analysis of complications arising from interruption is reviewed elsewhere in this issue (see "Complications of HIV Disease and Antiretroviral Therapy"), and selected trials on outcomes are below.

The Experienced (E)-184V study (Abstract 516) is a prospective, open-label study. Patients in whom lamivudine-containing antiretroviral regimens were failing and who had documented M184V mutations, CD4+ counts above 500 cells/ μ L, and plasma HIV-1 RNA levels above 1000 copies/mL, and who requested treatment interruption, were randomized to either treatment interruption or lamivudine 300 mg daily as monotherapy. Data were presented for 144 weeks and the 29 patients in each group did not differ in baseline parameters, including CD4+ count (566 cells/ μ L in the treatment interruption group vs 580 cells/ μ L in the lamivudine monotherapy group) or HIV-1 RNA level ($3.7 \log_{10}$ copies/mL

in the treatment interruption group vs $3.8 \log_{10}$ copies/mL in the lamivudine group). Time to immunologic or clinical failure (defined as CD4+ count below 350 cells/ μ L or Centers for Disease Control and Prevention [CDC] class B or C diagnosis) took a median of 20 weeks (interquartile range [IQR], 16–84 weeks) for the treatment interruption group and 84 weeks (IQR, 36–144 weeks) for the lamivudine monotherapy group. In analysis of variance (ANOVA), the treatment interruption group had greater declines in CD4+ count over time than the lamivudine monotherapy group ($P = .0095$), as did patients with baseline CD4+ counts of 700 cells/ μ L or lower compared with patients with baseline CD4+ counts of greater than 700 cells/ μ L ($P < .0001$). The plasma HIV-1 RNA trend did not differ statistically significantly over time between the 2 groups. By 144 weeks, 93% of patients in the treatment interruption group and 90% of patients in the lamivudine monotherapy group had discontinued the study. There were more grade III and IV adverse events in the treatment interruption group than in the lamivudine monotherapy group (8 vs 1; $P = .012$). Lamivudine monotherapy in this study of treatment-experienced patients led to persistently better clinical and virologic outcomes than complete treatment interruption.

Watts and colleagues (Abstract 751) examined the effects of treatment interruption after pregnancy in the Women and Infant Transmission Study (WITS) cohort. The 206 women included in the analysis were antiretroviral-naïve, had CD4+ counts above 350 cells/ μ L, and were similar in baseline characteristics to the larger WITS cohort. One hundred and forty-seven women continued antiretrovirals post-partum, and 59 stopped therapy. Those continuing therapy were slightly older (mean ages 27.7 years vs 25.9 years; $P = .04$) and a smaller percentage of them had CD4+ counts above 500 cells/ μ L (54.4% vs 71.2%; $P = .03$). Neither the slopes of the CD4+ cell count changes, plasma HIV-1 RNA levels, nor the number of class B events differed between the 2 groups during the first

postpartum year. Although the data are reassuring that stopping antiretroviral therapy after delivery does not lead to a more rapid decline in CD4+ count, studies with larger sample sizes and longer follow-up periods are needed.

Interleukin-7-based Therapies

Interleukin-7 (IL-7) is a cytokine that induces T-cell development, homeostasis, thymopoiesis, and T-cell maturation. It has been shown to cause dose-dependent increases in T cells in patients undergoing chemotherapy for cancer.

In an evaluation the safety and biologic effects of recombinant human IL-7 in patients chronically infected with HIV-1 (Abstract 127), 6 patients with CD4+ counts between 100 and 400 cells/ μ L and plasma HIV-1 RNA levels below $1.7 \log_{10}$ copies/mL received 8 subcutaneous injections of 3μ g/kg IL-7 over 18 days. Plasma HIV-1 RNA levels remained below $1.7 \log_{10}$ copies/mL for all patients and no biologic adverse effects above grade II were observed. The median CD4+ count increased from 210 cells/ μ L at baseline to 405 cells/ μ L at day 21 and remained above baseline at 300 cells/ μ L at week 12 ($P < .01$). An expansion of CD8+ cells expressing CD28 was also noted, suggesting that IL-7 may promote CD8+ cell maturation in vivo. The trial continues to examine the efficacy and safety of higher doses of IL-7.

Sereti and colleagues (Abstract 128) report the results of ACTG A5214, a randomized, placebo-controlled, double-blind phase I dose escalation study of IL-7. Sixteen participants (12 active, 4 placebo) with a median CD4+ count of 601 cells/ μ L and plasma HIV-1 RNA level of below $1.7 \log_{10}$ copies/mL were stratified by plasma HIV-1 RNA into 1 of 2 groups, below $1.7 \log_{10}$ copies/mL or 1.7 to $4.7 \log_{10}$ copies/mL. They were then randomized in a 3-to-1 fashion to receive one dose of 3, 10, 30, or 60 μ g/kg of IL-7 or placebo subcutaneously. Two dose-limiting toxicities were seen at the 60 μ g/kg dose, therefore the maximum tolerated dose was set at 30 μ g/kg. For the placebo group, no statistically significant increase in

within-subject change in CD4+ count from baseline was seen at day 1, 4, 14, or 28. For the IL-7 group, a statistically significant within-subject CD4+ count change from baseline occurred at day 1 (decrease of 426 cells/ μ L; $P = .423$) and day 14 (increase of 186 cells/ μ L; $P = .04$). At day 28, the within-subject CD4+ count in the IL-7 group had increased by 213 cells/ μ L, but this was not statistically significant.

Antiretroviral Therapy in Resource-limited Settings

One of the most exciting aspects of this year's conference was the increasingly global nature of the event. Several plenary sessions and many abstracts presented data from resource-limited settings, and the level of commitment to HIV/AIDS treatment in resource-limited settings was inspiring. Results from selected studies in resource-limited settings are summarized in Table 3.

Adult Treatment Outcomes in Large Cohorts

Matthias Egger delivered a plenary talk (Abstract 62) entitled "Outcomes of Antiretroviral Therapy in Resource-limited Settings." He pooled data from several sources, including the Antiretroviral Therapy Cohort Collaboration (ART-CC), a network of European and North American cohorts, and the International Epidemiological Databases to Evaluate AIDS (IeDEA), a collaborative effort to establish regional networks of treatment sites throughout Africa, Latin America, and Asia. Using data from 33,008 treatment-naïve patients across 42 countries and 176 treatment sites, Egger and colleagues determined that the median CD4+ count in selected countries at initiation of therapy ranged from 164 to 187 cells/ μ L in North America, 87 to 125 cells/ μ L in sub-Saharan Africa, and 53 to 206 cells/ μ L in Asia. Although the trend in median CD4+ count increased from 2001 to 2005 in sub-Saharan Africa, it remains significantly lower than in European and North American cohorts. The investigators found that all areas except for Western Europe used

a combination of 2 nRTIs and a NNRTI as the most frequent first-line regimen. However, the number of possible first-line regimens used to treat 90% of antiretroviral-naïve patients is 59 in North America, 47 in Western Europe, and 3 in all regions of Africa and Asia.

In an analysis of loss to follow up, Egger presented data from the Antiretroviral Therapy in Lower Income Countries (ART-LINC) collaboration on 16 treatment programs in resource-limited settings, 12 of which use active tracing of patients. He included 5575 adult patients (46% women) initiating antiretroviral therapy with a median age of 35 years, and found that 4% of patients did not return after their first visit and 17% were lost to follow up after the first 6 months. The HR for loss to follow up increased in each calendar year since 2000. In 2001 and 2002 the HR was 2.77 (95% CI, 1.69-4.55) and in 2003 and 2004 it was 7.86 (95% CI, 4.71-13.1). Patients with CD4+ counts below 50 cells/ μ L were also more likely to be lost to follow up, highlighting the need for active follow up in the determination of mortality estimates. Curves for virologic response, defined as plasma HIV-1 RNA below 2.7 \log_{10} copies/mL, were developed using data from the Swiss HIV Cohort and the Gugulethu and Khayelitsha township cohorts in South Africa; responses were similar between the cohorts, as were the rates of virologic rebound. In contrast, rates of treatment change varied dramatically between the cohorts: at 24 months approximately 60% of the Swiss cohort had changed regimens, compared with approximately 35% of the South African cohorts. The majority of this difference was attributed to changes for toxicity and by patient request, not for treatment failure.

An analysis of the 4 sub-Saharan Africa cohorts in the IeDEA collaboration and the ART-CC data revealed that tuberculosis (TB) is currently the most common opportunistic infection in both resource-limited and industrialized settings, although the incidence of TB is much higher in sub-Saharan Africa (approximately 250 cases per 1000 person-years) than in Europe and North America (approximately 25

cases per 1000 person-years). Crude mortality at 4 years was approximately 15% in sub-Saharan Africa, compared with approximately 5% in North America and Europe. A rapid increase in cumulative mortality in the first few months of treatment in sub-Saharan Africa was not observed in the ART-CC cohort. Breaking down mortality rates within the first year of treatment by cohort, and adjusting for baseline age, sex, CD4+ cell count, year, and disease stage, a wide range of mortality was observed in the North American and European cohorts, some with rates of 4% to 6%, similar to those observed in sub-Saharan Africa. Egger concluded that many patients in resource-limited settings are starting antiretroviral therapy later than recommended, but that virologic and immunologic responses are similar across regions. He highlighted the problem of loss to follow up in programs, which likely leads to underestimates of mortality, and the need for continued monitoring in the setting of rapid scale-up in resource-limited settings. Finally, he noted that although mortality rates are higher, particularly in the first few months of treatment, it is possible to achieve mortality rates in resource-limited settings that are comparable with some cohorts in North America and Europe.

El-Sadr and colleagues (Abstract 534) presented data on 171,259 patients receiving care from International Center for AIDS Care and Treatment Programs (ICAP)-sponsored sites in 7 different African countries, 71,482 of whom initiated potent antiretroviral therapy between July 2004 and December 2006. The investigators noted wide variations in populations, treatment outcomes, loss to follow up, and mortality among patients enrolled at each treatment site. Of the 116,609 patients who received HIV care from October 2006 to December 2006, 15% were eligible for antiretroviral treatment. Initiation of therapy varied from site to site, with 47% of eligible patients initiating antiretroviral therapy within 3 months in South Africa compared with 100% of eligible patients in Rwanda. Baseline CD4+ counts were low and ranged from 104 to 198 cells/ μ L. Most adult patients ini-

tiated treatment with stavudine/lamivudine/nevirapine, except in South Africa, where stavudine/lamivudine/efavirenz was the most common regimen. After 12 months on therapy, the average median CD4+ count was 291 cells/ μ L, and in the overall cohort 98% of adults and 93% of children remained on first-line regimens. The proportion of patients known to have died without active case-finding ranged from 5% to 6% when averaged by country, but some sites in Ethiopia and Mozambique reported that more than 15% of patients on antiretrovirals were known to have died. The proportion of patients lost to follow up without active case-finding ranged from 1% in Rwanda to 17% in Kenya.

The importance of active case-finding of patients who are lost to follow up was highlighted in a retrospective cohort study among 410 HIV-1-infected adults consecutively presenting to an urban clinic in Botswana (Abstract 537). Standard of care in the clinic was active case-finding for all patients lost to follow up and involved telephone calls and home visits if needed. Patient outcomes were retrospectively classified by passive case-finding, in which patients were classified as dead if their death was recorded in the clinic chart, and lost to follow up if their last clinic contact was more than 30 days past their last visit. Median duration of follow up was 44 weeks among the 410 patients initiating antiretroviral therapy during the study period. Passive case-finding classified 29 patients (7%) as dead and 68 of 410 patients (17%) as lost to follow up. Active case-finding classified 69 patients as dead (17%) and 22 (5%) as lost to follow up. The 52-week Kaplan-Meier survival estimates differed significantly: 0.93 (95% CI, 0.88-0.94) for passive and 0.79 (95% CI, 0.74-0.81) for active case-finding. Baseline CD4+ count below 100 cells/ μ L and male sex were independently associated with death in the first 12 months of antiretroviral therapy.

Nachegea and colleagues (Abstract 33) examined the effectiveness of efavirenz- and nevirapine-based antiretroviral regimens in a cohort of 2821 patients on antiretroviral therapy in 9 Southern African countries between January 1999 and March 2003. Mean age was 37

years and median follow-up time was 2.2 years. All patients initiated first-line antiretroviral treatment; 64.6% received efavirenz-based regimens (60% female) and 35.4% received nevirapine-based regimens (68% female). The baseline mean CD4+ count was 146 cells/ μ L for the efavirenz group and 167 cells/ μ L for the nevirapine group, and plasma HIV-1 RNA level was above 5.0 log₁₀ copies/mL for 61% and 55% of the efavirenz and nevirapine groups, respectively. CD4+ count outcomes data were not presented, but in a multivariate analysis controlling for adherence and other baseline variables, the HR for time to virologic failure after initial suppression was 0.72 (95% CI, 0.59-0.88) for efavirenz compared with nevirapine. Low adherence based on pharmacy claims data, low baseline CD4+ count, and high baseline plasma HIV-1 RNA correlated with decreased time to virologic failure.

Determinants of mortality were evaluated in a cohort of 1120 antiretroviral-naïve patients enrolled in a home-based treatment program in Uganda (Abstract 34). Median age was 38 years, 73% were female, and 39% were WHO stage III or IV when they initiated therapy. Median baseline CD4+ count and HIV-1 RNA level were 127 cells/ μ L and 5.3 log₁₀ copies/mL, respectively. Early mortality, defined as death occurring within 3 months of treatment initiation, was 16.4 per 100 person-years of observation in the cohort. Mortality decreased in each time period to a low of 1.3 per 100 person-years of observation at 18 to 24 months on treatment. Baseline factors associated with mortality were low CD4+ count, hemoglobin less than 10 g/dL, body mass index (BMI) less than 18 kg/m², and a history of prior TB. Adherence to antiretroviral treatment strongly correlated with mortality in reported adherence of less than 90% in the first 6 months (HR, 3.3; *P* < .001) and after the first 6 months (HR, 7.4; *P* < .001). The most common conditions associated with death in first 3 months on treatment were no diagnosis, TB, cryptococcal disease, and oropharyngeal candidiasis.

Van Custem and colleagues (Abstract 535) presented 5 years of data

from one of the longest-running treatment cohorts in a resource-limited setting, the Khayelitsha township program in Cape Town, South Africa. A total of 2565 antiretroviral-naïve patients initiated therapy between 2001 and mid-2006, 70% of whom were female. Median CD4+ count at treatment initiation increased over time from 44 cells/ μ L in 2001 to 2002, to 99 cells/ μ L in 2005. After 3 years of antiretroviral therapy, median CD4+ count was 422 cells/ μ L and plasma HIV-1 RNA was below 2.6 log₁₀ copies/mL in 80% of the patients. Mortality at 6 months after antiretroviral initiation decreased each year, and was 12.4% in 2001 and 5.4% in 2005. Determinants of mortality included an initial CD4+ count of less than 50 cells/ μ L, WHO stage IV disease, and a history of or current Kaposi's sarcoma (KS). At 48 months on treatment 14% had started second-line therapy, and the proportion of patients remaining in care at 54 months (combining mortality and loss to follow up) was 78%.

Two abstracts presented data on responses to second-line therapy in resource-limited settings from Médecins Sans Frontières-supported programs. The first, presented by Calmy and colleagues (Abstract 35), detailed outcomes from 22 countries in Africa, Asia, and Central America. Of more than 80,000 antiretroviral-naïve patients first initiating antiretroviral therapy since 2001 at a Médecins Sans Frontières site, 354 (0.4%) had changed regimens. The median age of those switching was 35 years, 57% were female, and 87% were WHO stage III or IV at therapy initiation. Stavudine/lamivudine/nevirapine was the initial regimen for 91% of the patients. Of the second-line regimens, 47% were nelfinavir-based and 46% were lopinavir/ritonavir-based. The median CD4+ count at switch was 99 cells/ μ L and the median plasma HIV-1 RNA level was 4.64 log₁₀ copies/mL for those programs with virologic testing. The median increase in CD4+ count was 91 cells/ μ L at 6 months and 113 cells/ μ L at 12 months. Approximately 6% were lost to follow up at 5 months, and 7% of the patients had died by 6 months. Probabilities of survival within the cohort, including death and loss to follow up, were 0.91 at 6 months

(IQR, 0.88-0.95 months) and 0.86 at 12 months (IQR, 0.82-0.91 months).

Ferradini and colleagues (Abstract 36LB) examined the efficacy of lopinavir/ritonavir-based second-line therapy at several Médecins Sans Frontières sites in Cambodia. One hundred and thirteen patients who had been followed up for at least 6 months on second-line therapy were identified; 50% were female and median age was 38 years. The decision to stop first-line therapy was based on immunologic and virologic criteria in 35 and 78 patients, respectively. Median CD4+ count at regimen switch was 70 cells/ μ L and median plasma HIV-1 RNA level was 4.7 \log_{10} copies/mL. The most frequent second-line regimens were didanosine/lamivudine/lopinavir/ritonavir ($n=47$) and didanosine/zidovudine/lopinavir/ritonavir ($n=21$), and the median duration of second-line therapy at the time of the evaluation was 10.2 months. Median CD4+ count increase was 105 cells/ μ L at 6 months and 180 cells/ μ L at 12 months. Plasma HIV-1 RNA at evaluation was below 2.6 \log_{10} copies/mL for 101 patients (89.4%), between 2.7 and 3 \log_{10} copies/mL for 6 patients (5.3%), between 3 and 4 \log_{10} copies/mL for 2 patients (2.7%), and more than 4 \log_{10} copies/mL for 4 patients (3.5%). Genotypic analysis in all patients with plasma HIV-1 RNA above 2.7 \log_{10} copies/mL did not reveal any protease mutations, but 100% had NNRTI resistance and 91.5% had an M184V mutation. Other than low CD4+ cell count at switch, there were no predictors of second-line therapy failure. The authors concluded that short-term outcomes of empiric, second-line lopinavir/ritonavir-based regimens were successful and that adherence, although not measured in this study, was a main determinant of second-line regimen failure.

Treatment Outcomes for Children in Large Cohorts

Kline and colleagues (Abstract 79) pooled database and medical record information for 11,926 children, including 5151 children receiving antiretroviral treatment through January 31, 2007, at 5 Baylor College of Medicine-supported

sites in Botswana, Uganda, Lesotho, Swaziland, and Malawi. Mean age at antiretroviral initiation ranged among sites from 5.1 to 7.8 years, 50% were female, and 50% to 92% had WHO stage III or IV disease. The vast majority of children were on first-line antiretroviral regimens, with 50% having received zidovudine/lamivudine/nevirapine. In the Botswana clinic, the median CD4+ cell percentage at baseline was 15, compared with 8 in Uganda. In Botswana ($n=880$), the CD4+ cell percentage was 27 at 6 months, 30 at 12 months, and 32 at 36 months. In Uganda, the CD4+ cell percentage was 18 at 6 months, 23 at 12 months, and 26 at 24 months. Plasma HIV-1 RNA data were available in the Botswana clinic, with levels of below 400 copies/mL in 79%, 81%, and 71% of patients at 6, 12, and 24 months, respectively. The crude mortality of the entire Botswana clinic population, both on and off antiretroviral therapy, decreased dramatically from 4.7% in 2004 to 0.3% in 2006. At 2.5 years of follow up the Botswana clinic had 10% of its patient population on second-line regimens and 93% still alive and on antiretroviral therapy.

Arrivé and colleagues (Abstract 727) presented data from 8 clinical centers participating in the KIDS-ART-LINC collaboration. Of 2142 children initiating antiretroviral therapy between 1 and 15 years of age, 53.5% were 5 to 15 years old, 16.5% were 36 to 59 months old, 20.9% were 12 to 35 months old, and 9.1% were less than 1 year old. Based on CD4+ cell percentage, 65.7% of the children met WHO criteria for severe immunodeficiency. PI-based regimens were used initially in 57.8% and NNRTI-based regimens in 37.0%. After 1 year of antiretroviral treatment without active case-finding, 4.4% were dead and 12.2% were lost to follow up. Estimated cumulative mortality was 5.5% (95% CI, 4.3-7.1) at 6 months and 6.5% (95% CI, 5.1-8.2) at 1 year. The probability of death varied dramatically by age group: 16.8% mortality (95% CI, 11.7-23.7) among children initiating antiretrovirals before 12 months of age compared with 4.0%, 1.8%, and 4.3% mortality for those initiating at 12 to 35 months, 36 to 59

months, and more than 60 months, respectively. Severe immunodeficiency and severe anemia were associated with increased risk of death.

Outcomes of 370 children receiving potent antiretrovirals with NNRTIs (48.6%) and PIs (51.4%) were compared in a clinic in Cape Town, South Africa (Abstract 728). The NNRTI group was 41% female compared with 53% female in the PI group ($P=.02$), and the median age was 34.3 months and 21.8 months in the NNRTI- and PI-based groups, respectively ($P<.01$). Duration on therapy was longer for the NNRTI group (3 years) than for the PI group (1.2 years; $P<.001$). Baseline CD4+ cell percentage was 13 and plasma HIV-1 RNA was 5.5 \log_{10} copies/mL for both groups. At 24 months there was no difference between groups in median CD4+ percentage (26.3) and survival curves were superimposable. However, plasma HIV-1 RNA levels differed, with 43% of the NNRTI group achieving virologic suppression at 24 months compared with 60% of the PI group ($P=.05$), and median plasma HIV-1 RNA was higher in the NNRTI group than in the PI group (3.8 \log_{10} copies/mL vs 2.6 \log_{10} copies/mL, respectively, $P=.05$). The authors speculated that the effectiveness of NNRTI-based regimens could be impaired by drug-drug interactions, suboptimal dosing, or pre-existing resistance as a result of prior exposure through prevention of mother-to-child transmission (PMTCT) programs.

Kamya and colleagues (Abstract 732) examined predictors of long-term virologic failure in a prospective cohort of 250 children and 526 adults initiating first-line antiretroviral treatment in Uganda between April 2004 and June 2005. The adult population was 69% female, had a mean age of 37 years, and 88% had WHO stage III and IV disease. The children were 48% female, had a mean age of 9.2 years, and 98% had WHO stage III and IV disease. All initiated NNRTI-based regimens, with stavudine/lamivudine/nevirapine being the most common in adults (73%) and zidovudine/lamivudine/efavirenz the most common in children (55%). Median CD4+ count was 99 cells/ μ L in

Table 3. Selected Studies from Resource-limited Settings

Study Name Abstract No.	Country, Treatment Program Duration of follow up	Baseline treatment regimen (No. Patients)	Baseline age, sex, clinical stage, treatment experience
Efavirenz- vs Nevirapine-based ART Regimens: Adherence and Virologic Outcomes Abstract 33	9 countries in southern Africa, Private-sector Aid for AIDS Disease Management Program Jan 1999-Mar 2003; median, 2.2 y	nRTI + efavirenz (64.6%) vs nRTI + nevirapine (35.4%) (n=2821)	37.0 y, efavirenz: 60% female, nevirapine: 68% female, antiretroviral treatment-naïve
Determinants of Mortality among HIV-infected Individuals Receiving Home-based ART in Rural Uganda Abstract 34	Uganda, Global AIDS Program Median, 2 y	Nevirapine/lamivudine/stavudine (96%) Efavirenz/lamivudine/stavudine (4%) (n=1120)	Median 38.0 y, 73% female, 39% WHO Stage III or IV, antiretroviral treatment-naïve
Variability in Populations Enrolled and Their Outcomes in HIV Care and Treatment Programs Across Countries in Sub-Saharan Africa Abstract 534	Ethiopia, Kenya, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, ICAP-supported, PEPFAR-funded Sep 2004-Jun 2006	Majority on stavudine/lamivudine/nevirapine, except South Africa: stavudine/lamivudine/efavirenz (86%) (n=116,284 total; 71,482 on antiretrovirals)	58% female (of those on antiretrovirals), antiretroviral treatment-naïve
Clinical Outcomes and Emerging Challenges after 5 Years of ART in a South African Township Abstract 535	South Africa, government-sponsored program 2001-mid-2006	Stavudine/lamivudine/efavirenz (37%) Stavudine/lamivudine/nevirapine (43%) (n=2565)	Median 32 y, 70% female, 90% WHO Stage III and IV, antiretroviral treatment-naïve
Field Effectiveness of HAART in HIV-infected Adults in West Africa: The Aconda/ISPED/EGPAF Program, Abidjan, Côte d'Ivoire 2004-06 Abstract 541	Côte d'Ivoire, PEPFAR-sponsored Mar 2004-Aug 2006	Stavudine/lamivudine/nevirapine (50%) Stavudine/lamivudine/efavirenz (22%) Zidovudine/lamivudine/efavirenz (21%) (n=7862)	34 y, 71% female, 77% WHO Stage III or IV, antiretroviral treatment-naïve
Catalyzing the Care and Treatment of HIV-infected Children in Sub-Saharan Africa: Early Outcomes from 5 Baylor College of Medicine Centers Abstract 79	Botswana, Uganda, Lesotho, Swaziland, and Malawi, joint Baylor, CDC, industry foundations, and government-sponsored Dec 2001-Jan 2007	First-line therapy with zidovudine/lamivudine/nevirapine (n=5151)	5.1-7.8 y (mean), 50% female, 50%-92% with WHO Stage III or IV, antiretroviral treatment-naïve
Outcomes of Adults Receiving Second-line ART in Médecins Sans Frontières-supported Projects in Resource-limited Countries Abstract 35	22 countries in Africa, Asia, and Central America, MSF-supported programs Median time from antiretroviral initiation to switch 20 mos, median follow-up post-switch was 7 mos	91% had first-line stavudine/lamivudine/nevirapine 47% had second-line nelfinavir-based, 46% had second-line lopinavir/ritonavir-based 59% had didanosine-containing (n=352, 0.4% of adults initiating antiretroviral treatment with MSF since 2001)	Median, 35 y, 57% female, at first-line antiretroviral initiation: 87% WHO Stage III or IV, included only patients entering program who were antiretroviral treatment-naïve

BMI indicates body mass index; CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; Hgb, hemoglobin; ICAP, International Center for AIDS Care and Treatment Programs; MSF, Médecins Sans Frontières; n.a., not available; PEP-

Baseline CD4+ cells/ μ L* Log ₁₀ Copies HIV RNA/mL*	CD4+ cells/ μ L * Response	Response in Plasma HIV RNA copies/mL*	Mortality	Comments
Efavirenz 146 (mean) Nevirapine 167 (mean) >5.0 efavirenz: 61% >5.0 nevirapine: 55%	Data not presented	In multivariate analysis controlling for adherence, hazard ratio for time to virologic failure after initial suppression was 0.72 (0.59-0.88) for efavirenz vs nevirapine	Data not presented	Low adherence, low baseline CD4+ count, and high baseline plasma HIV RNA levels correlate with decreased time to virologic failure Results could be due to efavirenz superiority or unmeasured confounders
127 (median) 5.3 (median)	Data not presented	Data not presented	Defined early mortality as \leq 3 mos, 16.4 per 100 person-years Mortality decreased in each time period to 1.3 per 100 person-years from 18-24 mos	Significantly more deaths from wasting in 0-3 mos Most common conditions contributing to death in first 3 mos were: no diagnosis, Tb, cryptococcal disease, o/p candida Baseline factors associated with mortality: CD4+ count 50-199 cells/ μ L, Hgb \leq 10g/dL, BMI < 18 kg/m ² , prior Tb, and depression index score
104-198 HIV RNA n.a.	At 6 mos: 246 (range, 223-290) At 12 mos: 291 (range, 277-305)	No data available	Mortality varied from 5% in Rwanda, Mozambique, and Tanzania to 15% in individual sites in Mozambique and Ethiopia (without active case finding)	The proportion of patients lost to follow up, without active case-finding, ranged from 1% in Rwanda to 17% in Kenya 98% of adult and 93% of children remained on first-line regimens
44 (median, 2001-2002) and 99 (median, 2005) HIV RNA n.a.	At 3 years: 422 (median)	At 3 years: 80% with <400	Mortality at 6 mos (year of antiretroviral start): 12.4% (2001) 5.4% (2005) Proportion remaining in care at 54 mos: 78%	Determinants of mortality: initial CD4+ count < 50 cells/ μ L, WHO stage IV disease, Kaposi's sarcoma at any point 14% started second-line therapy by 48 mos
116 (median) HIV RNA n.a.	At 6 mos: +136 At 12 mos: +163 At 18 mos: +213 (in those still on treatment)	No data available	12-mo survival probability (baseline CD4+ count): 77% (\leq 50 cells/ μ L) 86% (51-100 cells/ μ L) 89% (101-150 cells/ μ L) 93% (>150 cells/ μ L)	Lost to follow up at 12 mos: 19% Factors associated with mortality: baseline CD4+ count <50 cells/ μ L, male sex, older age, low baseline Hgb, baseline BMI <18.5 kg/m ² , baseline WHO stage III or IV, care center
Botswana: CD4+ percentage: 15 0%<400 copies/mL Uganda: CD4+ percentage: 8 HIV RNA n.a.	Botswana: (n=880) at 6 mos: +27% at 12 mos: +30% at 36 mos: +32% Uganda: at 6 mos: +18% at 12 mos: +23% at 24 mos: +26%	Botswana: at 6 mos: 79% <400 at 12 mos: 81% <400 at 36 mos: 71% <400	Botswana: crude mortality of entire clinic (on and off antiretrovirals): 2003: 4.7% 2004: 2.1% 2005: 1.1% 2006: 0.3%	Botswana at 2.5 y: 10% switched to second or third-line treatment 93% alive and on treatment
At first-line antiretroviral initiation: 63 (median), and at antiretroviral switch: 99 (median) 4.64 (for those available)	At 6 mos: + 91 (median) At 12 mos: +113 (median)	No data available	At 6 mos: ~7% died Probabilities of survival (death plus lost to follow-up): At 6 mos: 0.91 At 12 mos: 0.86	At 5.0 mos: 6% lost to follow up Factors associated with progression to death: CD4+ nadir <50 cells/ μ L Authors' speculation: low rate of second-line therapy use may be due to lack of availability, provider hesitation due to clinical status

FAR, United States President's Emergency Plan for AIDS Relief; TB, Tuberculosis; WHO, World Health Organization. *CD4+ counts and HIV RNA are in units stated unless indicated otherwise.

adults and 272 cells/ μ L (CD4+ cell percentage, 8.6) in children; mean plasma HIV-1 RNA level at baseline was 5.3 log₁₀ copies/mL in both children and adults. Nevirapine or zidovudine had been used for PMTCT in 21 of the adults and 9 children. Children were almost twice as likely as adults to have plasma HIV-1 RNA above 2.6 log₁₀ copies/mL (26% vs 14%, respectively; $P = .0001$). Predictors of virologic failure in children were male sex (odds ratio [OR], 2.54; 95% CI, 1.18-5.57), CD4+ percentage below 5 (OR, 3.99; 95% CI, 1.75-9.07), and initial antiretroviral regimen of stavudine/lamivudine/nevirapine versus zidovudine/lamivudine/efavirenz (OR, 3.33; 95% CI, 1.51-7.36). The same initial antiretroviral regimen predicted failure in adults.

Nkengasong and colleagues (Abstract 729) analyzed data from 134 children receiving their first antiretroviral treatment regimen between August 1998 and September 2003 in Côte d'Ivoire. At baseline, the median age was 7 years, 80% of the children had a CD4+ percentage of below 15, and the median plasma HIV-1 RNA level was 5.6 log₁₀ copies/mL. After 1 year of antiretroviral therapy, 54% of children had an undetectable plasma HIV-1 RNA and cumulative probability of developing any class of drug resistance was 0.44 (95% CI, 0.35-0.53). The magnitude of the virologic response was associated with emergence of drug resistance, as were smaller increases in CD4+ count from baseline, and dual-drug regimens.

Adherence in Resource-limited Settings

Although the need for strict adherence to antiretroviral medications is well documented in the literature, factors affecting antiretroviral treatment discontinuation and modification in resource-limited settings are not well described. Three abstracts took different approaches to adherence evaluation in Africa.

Kiguba and colleagues (Abstract 530) conducted a cross-sectional study of 686 individuals on antiretroviral

therapy in 2 treatment centers in Kampala, Uganda. The median age of participants was 36 years, median CD4+ count was 175 cells/ μ L, and 70% were female. The majority of the patients (83.8%) were receiving NNRTI-based regimens. Adherence was assessed by self-report using semistructured quantitative and unstructured qualitative interviews. Ninety-four patients (13.7%) had at least 1 episode of discontinuation (defined as simultaneous discontinuation of all antiretroviral drugs for at least 1 month). There were 175 patients (25.5%) who reported modifying therapy by changing or switching at least 1 antiretroviral medication. The most common reason for discontinuation was drug cost (43%), and for modification was avoidance of adverse events (71.8%). In a multivariate logistic regression analysis adjusting for baseline parameters, factors associated with discontinuation were duration of antiretrovirals of less than 1 year (OR, 11.11; 95% CI, 5.00-25.00), year of initiation being 2004 or earlier (OR, 4.42; 95% CI, 1.90-10.47), prior antiretroviral experience (OR, 3.70; 95% CI, 2.13-6.25), history of hospitalization (OR, 2.36; 95% CI, 1.32-4.20), and use of alternative medicines (OR, 2.18; 95% CI, 1.06-4.47). Modification was associated with duration of therapy more than 3 months (OR, 3.13; 95% CI, 1.16-8.33), year of initiation being 2004 or less (OR, 2.10; 95% CI, 1.02-4.31), being married (OR, 0.61; 95% CI, 0.37-0.98), and low regimen pill burden (OR, 0.04; 95% CI, 0.02-0.08).

Mosoko and colleagues (Abstract 536) used a retrospective cohort analysis to examine adherence to antiretroviral therapy and loss to follow up during 2 time periods in Limbe, Cameroon. The investigators compared data for patients before and after October 2004, when the government reduced the cost of antiretrovirals from approximately US \$30 per month to US \$6 per month. The annual gross domestic product per capita in Cameroon is US \$2400 (2006 estimate), but the population served by the clinic is highly economically disadvantaged and costs for antiretroviral therapy remain a large portion of household income even after the price reduction. A total of 2920 patients were

included in the study; the median age was 35 years, 62% were female, and the median follow-up time was 6.2 months. Some 55.7% had "good" access to the clinic, defined as living less than 40 kilometers away. Probability of remaining alive and in care at 15 months without active case-finding was not statistically significantly different between the 2 time periods (HR, 1.1; 95% CI, 1.0-1.2). Multivariate analysis revealed several factors associated with remaining in care, including female sex (HR, 1.2; 95% CI, 1.1-1.3; $P = .003$), good access to the clinic (HR, 1.5; 95% CI, 1.3-1.8; $P < .001$), and treatment paid by a funding program or employer (HR, 3.6; 95% CI, 2.2-6.0; $P < .001$). The investigators found that mean enrollment per month increased significantly with the decrease in antiretroviral cost: 46.5 persons enrolled per month in the first time period and 95.5 persons per month in the second ($P < .001$).

Nachegea and colleagues (Abstract 548) performed an analysis of direct health care costs associated with patient demographic characteristics, baseline CD4+ cell count, and level of antiretroviral adherence as determined by pharmacy claims data in 5455 HIV-1-infected adults initiating NNRTI-based antiretroviral therapy in southern Africa between 1998 and 2003. The cohort had a mean age of 37.1 years, and was 59.6% female. Mean baseline CD4+ count was 152.6 cells/ μ L and the mean follow-up period was 27.3 months. The investigators noted a statistically significant dose-response relationship between nonantiretroviral therapy-related health expenditures (eg, consultations, hospitalizations, investigations, and medications other than antiretrovirals) and adherence. In those patients whose adherence rate was 95% or higher, nonantiretroviral therapy costs were US \$152 per month (95% CI, 146-157), and in those with lower than 50% adherence, costs were US \$200 per month (95% CI, 189-211). Total monthly health care costs decreased with time on antiretroviral therapy by approximately US \$9 per month. Poor adherence, low baseline

CD4+ cell count, older age, and black race were all associated with higher total expenditures.

Laboratory Monitoring in Resource-limited Settings

Plasma HIV-1 RNA measurements are frequently unavailable in resource-limited countries and low-cost surrogates for virologic response would be a great benefit to monitoring antiretroviral therapy in these settings. In an attempt to predict virologic failure with CD4+ count change, Wood and colleagues (Abstract 538) examined a cohort of 161 patients receiving antiretroviral therapy in Capetown, South Africa, who had experienced at least 1 episode of virologic failure, defined as a single episode of plasma HIV-1 RNA above $3.0 \log_{10}$ copies/mL following a first plasma HIV-1 RNA of below $2.6 \log_{10}$ copies/mL. Risk of virologic failure was independently associated with baseline CD4+ count (relative risk [RR] 2.48; 95% CI, 1.07-5.74, $P = .04$) and with CD4+ count increases of less than 100 cells/ μ L during follow up (RR, 2.54; 95% CI, 1.01-6.43, $P = .03$). However, a negative CD4+ count slope in values 3 to 2 months prior to failure had a sensitivity for detecting virologic failure of only 55.3% (95% CI, 47.2-63.1), a specificity of 61.5% (95% CI, 59.6-63.3), a positive predictive value of 7.8% (95% CI, 6.3-9.5), and a negative predictive value of 95.9% (95% CI, 94.8-96.8) indicating that change or negative slope in CD4+ cell count was a poor predictor of virologic failure.

Meya and colleagues (Abstract 531) expanded on the above strategy in a cross-sectional study of 496 patients in Uganda on NNRTI-based antiretroviral regimens. They searched for treatment, adherence, clinical, and laboratory parameters that could predict virologic failure, defined as a plasma HIV-1 RNA level above $2.6 \log_{10}$ copies/mL. The cohort was 65% female and had a median age of 38 years, and 63% were on a nevirapine-based regimen. One hundred and seventeen patients had plasma HIV-1 RNA above $2.6 \log_{10}$ copies/mL. Predictors of vi-

rologic failure in multivariate analysis were CD4+ count below baseline with a fall of greater than 30% from the peak value achieved on antiretroviral treatment (OR, 3.7; 95% CI, 1.4-9.4, $P = .007$) and any treatment interruption of more than 2 days (OR, 3.6; 95% CI, 1.7-7.4, $P = .001$). A failure score was then developed using these 2 parameters with 1 point for each parameter. If both parameters were absent (score = 0) the test had a sensitivity of 57% (95% CI, 42-71), specificity of 81% (95% CI, 77-84), a positive predictive value of 25% (95% CI, 17-34), and negative predictive value of 95% (95% CI, 91-96) for predicting plasma HIV-1 RNA below $2.6 \log_{10}$ copies/mL. Applying this monitoring algorithm to the cohort, 112 patients would have had a failure score of 1 or 2 and been assigned to virologic testing, and 384 would have been assigned as score of 0 and not tested, resulting in 21 missed failures.

Iqbal and colleagues (Abstract 673) compared the performance of a non-nucleic acid-based plasma HIV-1 RNA assay to a gold standard nucleic acid-based assay on 121 plasma specimens from 107 HIV-1 subtype C-infected patients. The sensitivity of the non-nucleic acid-based assay to detect HIV-1 RNA below $2.6 \log_{10}$ copies/mL (equivalent to < 5500 copies/mL) was 100%. The difference seen in the non-nucleic acid-based assay compared with standard polymerase chain reaction (PCR) was a decrease of $0.23 \log_{10}$ copies/mL (95% CI, -0.91 - 0.45). This study suggested that this less expensive and technically simpler method should be evaluated further as a substitute for current plasma HIV-1 RNA tests.

Waters and colleagues (Abstract 674) conducted an analysis of filter paper transfer of whole blood and plasma samples from resource-limited settings to a European laboratory for plasma HIV-1 RNA determinations in patients on antiretroviral therapy. Blood samples from 402 patients in Uganda underwent local testing using a standard reverse transcriptase PCR-based assay. Blood droplets were simultaneously transferred to filter pa-

per that was sent to Holland every 3 weeks for RNA extraction and testing using a real-time reverse transcriptase PCR assay with a lower limit of detection of $2.7 \log_{10}$ copies/mL. Using the local testing as the gold standard, the whole-blood filter-paper transfer assay had a sensitivity of 86% (99% CI, 67-100), a specificity of 77% (99% CI, 69-85), a negative predictive value of 27% (99% CI, 14-40), and a positive predictive value of 98% (99% CI, 95-100). The plasma filter paper transfer assay showed improved performance, with a sensitivity of 100% (99% CI, 84-100), specificity of 99% (99% CI, 97-100), negative predictive value of 95% (99% CI, 77-99) and positive predictive value of 100% (99% CI, 98-100) compared with the local gold standard. Filter paper transfer of plasma specimens could be a reliable means of virologic testing in resource-limited settings. Whole-blood filter-paper transfer testing was limited by a high number of false-positive results.

Dried blood spots are used in resource-limited settings as an alternative to plasma for drug resistance testing. Masciotra and colleagues (Abstract 629) evaluated the level of concordance between resistance detected in plasma versus dried blood spot. Specimens from 60 patients infected with HIV-1 subtype B virus were collected, and successfully RNA genotyped in 50 patients. There was good correlation between plasma and dried blood spot identification of resistance mutations with dried blood spot detecting 97% (306 of 316) of mutations identified in plasma, and plasma detecting 95% (306 of 322) of mutations identified in dried blood spot. A majority of discrepancies were secondary to mixtures containing minor protease position alterations and unusual amino acids substitutions in reverse transcriptase. Only 2 major mutations were absent in dried blood spots (M46L and K103N). In this small study, dried blood spots appeared to be a feasible and reliable alternative to plasma for resistance mutation detection in resource-limited settings (see also⁵).

Prevention of Maternal-to-child Transmission

Bulterys presented an overview regarding the status of PMTCT and the reasons it continues to be inadequate in resource-limited settings (Abstract 11). Rates of maternal-to-child transmission (MTCT) of HIV without intervention are 15% to 45% but can be reduced to 1% using antiretroviral prophylaxis, caesarian delivery, and avoidance of breastfeeding. In industrialized nations, these interventions have been successful at reducing rates of MTCT to around 2% or less but in resource-limited settings, particularly sub-Saharan Africa, rates of MTCT remain close to rates without intervention. According to United Nations International Children's Emergency Fund (UNICEF) global estimates for 2005, there were 21 million HIV-seropositive pregnant women worldwide, of whom only 22% were identified as infected during pregnancy or delivery; 10% of mothers received antiretroviral prophylaxis and 8% of HIV-exposed infants received antiretroviral prophylaxis. Bulterys suggested that PMTCT continues to fail in resource-limited settings. Lack of resources is a key limitation to successful PMTCT, and includes inadequate antenatal care infrastructures, limited availability of rapid HIV test kits and antiretroviral medications, a disconnection between PMTCT programs that identify HIV-seropositive pregnant women and their families and programs that provide long-term HIV care and antiretroviral therapy, and competing health priorities in the face of decreasing health care resources. Lack of access to clean water and good alternatives to breastfeeding counteract decreased rates of HIV transmission in resource-limited settings due to increased rates of mortality in formula-fed infants (see "HIV Epidemiology and Prevention Interventions" in this issue). Bulterys concluded that the United Nations goal to decrease new HIV infections globally by 50% can be met but only by radically increasing access to and implementation of PMTCT. He suggested that full integration of maternal and

child health care, incorporation of non-professional trained birth attendants into PMTCT programs, recognition of PMTCT as a crucial entry point to care for HIV-seropositive women and their families, and improvement of linkages between PMTCT and long-term antiretroviral services are key strategies to decrease rates of MTCT. The following abstracts reported on current efforts and barriers to decreasing MTCT.

Antiretroviral Prophylaxis for Prevention of Mother-to-Child Transmission Among Women Ineligible for Antiretroviral Treatment

In the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) protocol, all HIV-seropositive pregnant women received potent antiretroviral therapy predelivery and postpartum regardless of virologic and immunologic status. In Abstract 747, outcomes of 341 HIV-seropositive women in Mozambique from the DREAM study who received triple-antiretroviral prophylaxis for PMTCT with either zidovudine/lamivudine/nevirapine or stavudine/lamivudine/nevirapine at 25 weeks gestation through 6 months postpartum were presented. At baseline, mean age, CD4+ count, viral load, and percent in clinical class WHO stage III to IV were 26 years, 422 cells/ μ L, 3.94 \log_{10} copies/mL, and 6, respectively. Median time of antiretroviral therapy predelivery was 87 days. At 1 month postpartum, 4 of 341 (1.2%) infants tested were HIV seropositive and at 6 months an additional 2 of 251 (0.8%) infants tested were HIV seropositive (98 infants were not yet 6 months old and 8 infants were lost to follow up). Seven infants died, all of whom were HIV seronegative at 1 month (mortality rate 28.5% child-years). Risk of MTCT was not associated with baseline CD4+ count or viral load. There was a trend of decreased transmission among women with longer predelivery exposure to antiretrovirals (129 days among nontransmitters vs 79 days among transmitters) but the difference was not statistically significant ($P = .58$).

In a cost-effectiveness analysis of

the DREAM study, costs for infections averted and Disability Adjusted Life Years (DALY) saved were calculated according to the Joint United Nations Programme on HIV/AIDS (UNAIDS) guidelines for intervention evaluation (Abstract 762). Of 6175 pregnant women who received antenatal HIV testing, 1862 tested HIV seropositive; 1594 of these HIV-seropositive pregnant women entered the program. The majority of program costs were spent on laboratory analyses (30%), medications (24%), and personnel (21%). Infection rates at 1 month and 6 months were 3.8% and 1.5%, respectively, resulting in an estimated 481 averted infections. The efficacy of the intervention was calculated to be 68.53% avoided infections through 6 months postpartum, with a calculated cost of US \$518 per infection averted and US \$22 per DALY saved. Subtracting the cost of care for HIV-seropositive children (US \$369 per HIV-seropositive child), cost per infection was US \$149 per infection averted and US \$6 per DALY saved. This study showed that PMTCT with potent antiretroviral prophylaxis is cost-effective. Additional benefits not reflected in this analysis include decreasing the number of orphans by increasing the life expectancy of the mother, supporting the health sector by training of local personnel, and decreasing stigma by improving the quality of life of HIV-seropositive adults and their families thereby facilitating other HIV and AIDS-related public health interventions.

Antiretroviral Treatment of HIV-seropositive Pregnant Women

Providing long-term potent antiretroviral therapy for eligible HIV-seropositive women is an important global public health initiative both for the women who require antiretroviral drugs and for PMTCT. The following studies evaluated outcomes among women who initiated antiretroviral therapy during pregnancy.

The DART study is a randomized trial of antiretroviral-monitoring strategies among adults with symptomatic HIV infection and CD4+ counts below 200 cells/ μ L in Uganda and Zimbabwe

(Abstract 746). Outcomes of 221 pregnancies in 198 women were assessed over a median follow-up period of 2.4 years. Median CD4+ count was 115 cells/ μ L and 18% were WHO Stage IV at baseline. Most of the women were on a regimen of zidovudine/lamivudine plus tenofovir (70%), nevirapine (15%), or abacavir (4%). Among the 164 women with a known outcome there were 91 live births (55%), 11 stillbirths (7%), and 62 terminations (38%). No infants were diagnosed with HIV infection. Four of the women died and 3 infants had congenital abnormalities. This is the largest data set on in utero exposure to triple antiretroviral therapy in resource-limited settings, the only data set evaluating in utero exposure to tenofovir to date, and is reassuring given the absence of perinatal transmission detected and rates of congenital abnormalities similar to other studies. Analysis of maternal and infant outcomes is ongoing.

A prospective study of HIV-seropositive, antiretroviral-naïve (aside from prior single-dose nevirapine for PMTCT) pregnant women with CD4+ counts below 350 cells/ μ L was conducted at a prenatal care clinic in Mozambique (Abstract 756). Antiretroviral therapy with nevirapine/lamivudine plus zidovudine or stavudine was initiated 3 weeks after enrollment and continued until at least 6 months postpartum. Infants were given single-dose nevirapine within 48 hours of delivery. Of 163 women who enrolled in the study, 148 received antenatal antiretroviral therapy and 146 were followed up through delivery. These 146 women delivered 149 infants, of whom 17 died prior to HIV testing and 26 were not tested. Seven of 106 (6.6%) tested infants were HIV-1 seropositive. Maternal and infant characteristics, including maternal baseline CD4+ count, HIV-1 RNA viral load, duration of antiretroviral therapy, and feeding strategy did not correlate with perinatal transmission. This relatively high rate of perinatal transmission in the setting of potent antiretroviral therapy could have resulted from subtherapeutic concentrations of antiretroviral drugs in the setting of pregnancy and nursing or

nonadherence, neither of which were assessed in this study.

Suppression of HIV-1 RNA During Prevention of Mother-to-Child Transmission

The European Collaborative Study is a cohort of HIV-1-infected pregnant women and their infants from 10 European countries (Abstract 758). An analysis of time to virologic suppression was conducted in 240 women from this cohort who had their initial diagnosis of HIV during pregnancy or documented nonreceipt of prior antiretroviral therapy. All women received potent antiretroviral therapy; 156 (65%) initiated a PI-based regimen (80% nelfinavir) and 84 (35%) initiated a nevirapine-based regimen. Fifty-nine percent were black, 90% were born in Africa, and 64% were diagnosed with HIV during pregnancy. At time of delivery, 73% achieved virologic suppression. Antiretroviral regimen did not correlate with virologic suppression but time to undetectable HIV-1 RNA was faster among patients on a nevirapine-based regimen (HR, 1.54), who had a country of origin in West Africa (HR, 1.90), and whose baseline HIV-1 RNA was below 3.81 log₁₀ copies/mL (HR, 2.76). Among women with baseline CD4+ count below 250 cells/ μ L, 82.4% on nevirapine-based regimens achieved virologic suppression at 8.5 weeks compared with 50.4% on PI-based regimens. Faster time to virologic suppression may have been due to the suboptimal efficacy of non-boosted PI nelfinavir pharmacokinetics during pregnancy leading to subtherapeutic levels, or nonadherence in the PI group, neither of which were assessed. Faster virologic suppression among women from West Africa may have been the result of differences in HIV-1 subtype or host biologic or genetic differences.

In a retrospective cohort of 114 women and infant pairs exposed to potent antiretroviral therapy in Vancouver, British Columbia (Abstract 759), 80% of women had achieved virologic suppression at time of delivery with no difference in probability of suppression between women on a PI-based regimen (n=57) versus an NNRTI-based regi-

men (n=34); 1 woman on a salvage regimen was not included. Women who had prior history of antiretroviral therapy had a longer time to achieving virologic suppression than did women with no prior history of antiretroviral therapy (58 vs 34 days, respectively). Adherence based on pharmacy records correlated with proportion of patients achieving virologic suppression: 90.2% of patients who had virologic suppression had "excellent adherence" compared with 54.5% of patients who did not achieve virologic suppression.

Antiretroviral Prevention of Mother-to-Child Transmission: Effects on Resistance in Mothers and Infants

Coffie and colleagues (Abstract 93LB) presented a prospective cohort study of 247 women in Côte d'Ivoire with at least 1 prior pregnancy who initiated antiretroviral treatment with stavudine or zidovudine plus lamivudine plus nevirapine or efavirenz. Virologic and immunologic responses were evaluated 12 months after initiation. Eighty-six women had previous exposure to single-dose nevirapine and short-course zidovudine with lamivudine for PMTCT, 52 had previous exposure to single-dose nevirapine and short-course zidovudine, and 109 women had no history of antiretroviral exposure for PMTCT. Of women who received nevirapine plus zidovudine/lamivudine, 11 of 73 had baseline resistance to lamivudine (15.1%) and 3 of 70 (4.3%) had baseline resistance to nevirapine. Sixteen of 42 (38.1%) in the nevirapine plus zidovudine group had baseline resistance to nevirapine. Neither group had evidence of zidovudine resistance. The overall rate of virologic failure (HIV-1 RNA > 500 copies/mL) at 12 months was 19% (42 of 219). Fifty percent of women with baseline lamivudine resistance had failure, compared with 18.9% of women exposed to lamivudine but with no evidence of baseline resistance. Among women with baseline nevirapine resistance 27.8% had failure, compared with 18.6% among women exposed to nevirapine but with no evidence of baseline nevirapine resistance. Women with no history of

antiretroviral exposure for PMTCT had a 16% rate of virologic failure. In multivariate analysis, baseline CD4+ count below 200 cells/μL, baseline lamivudine resistance and, especially, poor adherence were associated with virologic failure with ORs of 0.34, 6.86, and 12.68, respectively. PMTCT-acquired lamivudine resistance was associated with poorer 12-month virologic outcomes. However, time of initiation and duration of antiretroviral therapy may have confounded the outcomes in the lamivudine group, as time to initiation of antiretroviral therapy post-PMTCT was shorter among the lamivudine-exposed group than in women who received PMTCT prophylaxis without lamivudine (median 15 months vs 28 months), and time of exposure to antiretrovirals during PMTCT prophylaxis was longer in the lamivudine-exposed group (median 56 days vs 30 days).

A study of infants born to HIV-seropositive women in Mozambique (Abstract 92) compared rates of NNRTI resistance among infants infected in utero (HIV-1-seropositive by PCR at birth up to 2 weeks postpartum) with rates among infants infected intrapartum or early postpartum (HIV-1-PCR-positive 2–8 weeks postpartum). Standard of care for PMTCT in this setting is initiation of potent antiretroviral therapy in all HIV-seropositive pregnant women with CD4+ counts below 350 cells/μL, and short-course zidovudine at 34 weeks gestation followed by single-dose nevirapine in the mother at labor and in the infant at birth for HIV-seropositive pregnant women with CD4+ counts above 350 cells/μL. Data for 330 infants who have enrolled thus far were presented. Twenty-two of 330 infants (6.7%) were infected in utero (7 have died, 8 are lost to follow up, and 7 continue follow up), 14 of 199 infants (7.0%) were infected peripartum (excluding infants who were HIV-1 seronegative at birth but status at 8-week follow up was unknown) and in 131 of 330, HIV status at birth was unknown. Six-month mortality of infants infected in utero and intra-peripartum was 6 of 16 (37.5%) and 0 of 6 (0%), respectively. Oligonucleotide ligation assay specific for detecting the NNRTI mutations K103N, Y181C, and G190A

was conducted in samples from 29 infants. Four of 16 infants infected in utero and 4 of 13 infants infected intra-peripartum had evidence of nevirapine resistance. The 2 infants infected in utero who had wild-type virus received single-dose nevirapine at birth but their mothers did not receive nevirapine. Infants infected in utero who had resistance mutations had a mixture of mutant and wild-type virus, whereas infants infected intra-peripartum had either 100% resistant virus or 100% wild type. The dichotomy of virus (all resistant or all wild type) among infants infected intra-peripartum might result in persistent nevirapine resistance among infants infected with mutant strains. In contrast, the mixture of resistant and wild-type virus in infants infected in utero could result in a better chance of reversion to wild type. The high mortality associated with in utero infection indicates a need for earlier treatment. However, the high rates of nevirapine resistance in this group would require a non-nevirapine-based regimen. This study is ongoing and will assess rates of MTCT through breastfeeding and whether resistance mutations fade over time.

Antiretroviral resistance in breast milk. In a study of PMTCT among 40 HIV-seropositive pregnant women in Mozambique who received zidovudine/lamivudine/nevirapine at 28 weeks gestation through 4 weeks postpartum, levels of HIV-1 RNA and resistance patterns were evaluated in the first week after delivery (Abstract 764). Major resistance to NNRTIs and lamivudine were present in plasma of 3 of 40 (7.5%) and 1 of 40 (2.5%) women, respectively. Similarly, NNRTI and lamivudine resistance mutations were present in virus in the breast milk of 7.5% and 5% of women, respectively. Although prevalence of resistance was similar in plasma and breast milk, patterns of resistance were different in these compartments indicating that although some HIV-1 in breast milk is derived from diffusion from plasma, it is likely that local viral production also contributes to the HIV-1 population present in breast milk. In plasma and breast milk there were no correlations between level of HIV-1 RNA or concen-

trations of antiretrovirals and presence of major resistance mutations. Duration of antiretroviral prophylaxis was different between patients with and without major mutations (119 days vs 62 days, respectively, $P = .0002$). Given the evidence that avoidance of breastfeeding for PMTCT is not a viable option for women in living in resource-limited settings (see “HIV Epidemiology and Prevention Interventions” in this issue), the utility of antiretroviral therapy to prevent postnatal transmission requires further evaluation.

Antiretroviral Drug Resistance

Transmitted Drug Resistance

Epidemiology of Transmitted Drug Resistance. Rates of transmitted drug resistance (TDR) in Europe, Australia, and the United States range from 11% to 15% among patients with primary HIV-1 infection and 7% to 11% among patients with newly diagnosed HIV-1 in whom time of infection is unknown (Abstract 60). Data from 11 states in the US Variant, Atypical, and Resistant HIV Surveillance group (VARHS) were analyzed to estimate the prevalence of TDR and distribution of subtypes (Abstract 648). Specimens from 3130 antiretroviral-naïve individuals, newly diagnosed with HIV-1 infection from March 2003 to October 2006 were analyzed, and 10.4% had virus with drug resistance mutations. Resistance to NNRTIs, nRTIs, and PIs were present in 6.9%, 3.6%, and 2.4%, respectively, and 1.9% had multi-class resistance. Predominant mutations were K103N for NNRTI (70.1%), M41L for nRTI (45.1%), and L90M for PIs (40.0%). Non subtype B or recombinant forms were found in 5.1% of patients.

Two large intervention studies that recruited HIV-seronegative MSM evaluated the prevalence of drug resistance among men who seroconverted during study participation. In the EXPLORE study (Abstract 650), men were randomized to a behavioral intervention to prevent HIV-1 acquisition. Two hundred and fifty-nine men seroconverted and 195 had genotypic

ing results available for analysis. A total of 15.9% (31 of 195) had resistance mutations, 3.6% had multi-class resistance, and 5 men had CXCR4-tropic virus. In multivariate analysis, there was no association between resistance and demographic, clinical, or risk-factor data. In the gp120 vaccine efficacy trial (in which gp120 was found ineffective in prevention of HIV infection), 5095 HIV-seronegative MSM and 308 women at high risk for HIV were enrolled, and 362 men and 6 women seroconverted during the study (Abstract 653). Two hundred and eighty-six samples were available for sequencing, of which 16% had at least 1 resistance mutation and 7% had multi-class resistance. In this study, having an HIV-seropositive partner (OR, 4.6; 95% CI, 1.08-5.2, $P = .03$), reporting unprotected anal sex (OR, 5.5; 95% CI, 1.3-14.9, $P = .02$) and marijuana use (OR, 4.0; 95% CI, 1.02-4.2, $P = .04$) were independently associated with TDR.

Nambiar and colleagues (Abstract 657) found that TDR among individuals with primary HIV-1 infection was associated with diagnosis of a sexually transmitted disease (STD) within 3 months of HIV diagnosis. The overall rate of TDR was 15% (28 of 185) and of the 124 individuals screened for an STD, 45% were diagnosed with an STD; 68% had TDR versus 31% with wild-type virus ($P = .03$). The authors concluded that this correlation between TDR and presence of an STD could have resulted from facilitated transmission of less fit virus through mucosal breakdown in the setting of STDs or poor drug adherence among HIV-seropositive individuals in the sexual networks of people engaging in high-risk sexual behavior.

An analysis comparing cohorts of early acute, HIV-1-infected, antiretroviral-naïve individuals in 2003 to 2004 and 2005 to 2006 in New York City showed a decrease in TDR from 24.1% (27 of 112) to 12% (13 of 108), $P = .02$ (Abstract 651). The reason for this decrease is unclear, although the authors hypothesize that better drug adherence with less viral breakthrough in the potential transmitter

population may be a factor. Sentinel-site surveillance in STD clinics and HIV testing facilities in San Francisco (Abstract 652) showed that rates of TDR remained stable from 2004 to 2006 with an overall rate of 13.7% (55 of 402). Whether these trends continue and will be similar in other cohorts requires further evaluation.

Natural History of Transmitted Drug Resistance.

In the setting of secondary drug resistance, it takes an average of 12 to 16 weeks for a population of predominantly mutant virus to convert to majority wild-type virus after removal of drug pressure. In the case of TDR, it takes an average of 3 years or more for reversion to wild type, as the mutant virus is not competing with existing wild-type strains. Evaluating a group of 14 acutely infected HIV-1-seropositive patients with TDR, Little and colleagues (Abstract 60) determined the mean time to first appearance of a wild-type and resistant mixture to be 103 weeks (about 2.0 years; 95% CI, 49-216 weeks). They subsequently evaluated mean time to last wild-type and resistant mixture (ie, no evidence of resistant virus) and found that 13 of 14 patients had pure resistant virus or persistence of mixture; time to complete reversion by population sequencing in the 1 patient that converted to wild-type virus was 2.7 years. These patients continue to be followed up, and at 4 years of follow up, many patients continue to have pure or mixed resistant virus. Mean replication capacity of the TDR virus was 87%, which was not statistically significantly different from reference wild-type virus. In this cohort, detection of wild-type virus after acquisition of TDR virus takes an average of 2 years and complete reversion to wild-type virus theoretically might never occur. The high fitness displayed by these TDR viruses may be due to selected transmission of more fit resistant variants.

Among patients enrolled in the Acute Infection and Early Disease Research Program (AIEDRP) DACS 003 study from June 1993 to January 2007, the rate of TDR among recently HIV-1-infected individuals was 10.2%

(93 of 913) (Abstract 60). There was no statistically significant difference in baseline HIV-1 RNA level in patients with TDR compared with patients with susceptible virus, but when stratified by resistance class, individuals with NNRTI resistance had a baseline HIV-1 RNA level of 0.4 \log_{10} copies/mL higher than patients with susceptible virus ($P = .003$). Patients with nRTI resistance had a mean baseline HIV-1 RNA level of 0.7 \log_{10} copies/mL lower than patients with susceptible virus ($P = .001$). There was a trend toward lower baseline HIV-1 RNA level in patients with PI-resistance mutations than in patients with susceptible virus but the difference was not statistically significantly different. These differences in viral load continued for patients with NNRTI- and nRTI-resistance mutations at 1 and 3 years of follow up but the PI trend disappeared. The clinical significance of these differences in viral load is unclear and needs further confirmation in larger cohort studies.

Seven hundred and ninety-six patients with recent HIV infection were enrolled in AIEDRP DACS 002 from 1995 to 2006 and received antiretroviral treatment within 7 months of the estimated date of infection (Abstract 60). Of these patients 84 had TDR, and, compared with patients with susceptible virus, there was no statistically significant difference in time to reach HIV-1 RNA levels below 50 copies/mL. However, when evaluated by class, individuals with PI-resistance mutations had a longer time to reach HIV-1 RNA levels below 50 copies/mL than patients with susceptible virus ($P = .002$). This was likely due to the fact that from 1996 to 2000, resistance testing was not routinely performed prior to antiretroviral therapy initiation and 50% of patients with baseline PI resistance were initiated on a PI-based regimen. Complete viral suppression failed to occur in 45% of patients (38 of 84), of whom 70% had fewer than 3 active antiretroviral medications in their regimen. The difference in time to suppression was likely related to whether antiretrovirals to which the virus is susceptible

were used. Given the relatively high rates of TDR in more developed settings, the authors emphasized the importance of performing baseline resistance testing on newly diagnosed, antiretroviral-naïve patients in these areas, reaffirming current consensus recommendations.

Low-frequency Resistance Variants.

Each HIV-infected patient is infected with a strain of HIV that, over time, develops into a swarm of viruses containing different polymorphisms (ie, intra-patient viral diversity). Some of these polymorphisms are a result of the natural evolution of wild-type virus, and other polymorphisms represent resistance mutations that carry clinical significance. Conventional bulk resistance testing detects resistant variants that occur at a frequency greater than 20% above “background” wild-type polymorphisms. Low-frequency resistance variants are populations of resistant virus that occur at a frequency that is below the level detectable by bulk sequencing but above the background wild-type polymorphisms. Many studies have documented the existence of these low-frequency resistance variants among individuals with virologic failure but have no evidence of resistance based on bulk-resistance testing (Abstract 61). Johnson and colleagues (Abstracts 61, 639) used a real-time PCR detection assay to assess the prevalence of resistance among antiretroviral-naïve patients who had no detectable resistance by conventional sequencing and to evaluate the level of baseline low-frequency resistance among antiretroviral-naïve patients with known resistance by conventional sequencing. They found that 15% (30 of 205) of patients with wild-type virus by conventional sequencing had at least 1 major mutation detected by the more sensitive assay, 2% of whom had dual-class resistance. In a separate cohort of patients, 7% (21 of 302) of patients with known baseline resistance gained resistance to another drug class, based on real-time PCR testing. Rates of triple-class resistance doubled in this group to 5%.

Paredes and colleagues compared the detection of M184V and D30N

resistance mutations using standard, population-based genotype-sequencing versus allele-specific PCR among 61 antiretroviral-naïve pregnant women enrolled in the WITS. M184V was detected 1.5 times more frequently using allele-specific PCR than using standard genotype sequencing (13.3% vs 8.8%, respectively); D30N was detected 3 times more frequently in using allele-specific PCR than using standard genotype sequencing (6.7% vs 2.2%, respectively).

Each of these studies supports the conclusion that low-frequency resistance mutations are missed by conventional sequencing, but do these low-frequency resistant variants carry clinical significance, as has been suggested in previous studies? Peuchant and colleagues (Abstract 666) evaluated the effect of resistance detected by conventional and sensitive resistance testing on virologic and immunologic outcomes. Of 172 antiretroviral-naïve, recent HIV-1 seroconverters, 9.3% had resistance to at least 1 class at baseline. Baseline resistance was related to a lower baseline HIV-1 RNA level (3.76 log₁₀ copies/mL vs 4.59 log₁₀ copies/mL for resistant and wild-type virus, respectively; $P = .002$), higher baseline CD4+ counts (557 vs 425 cells/μL for resistant and wild-type virus, respectively; $P = .03$) and a less steep decrease in viral load after 1 month of treatment. In a substudy of 78 patients, they found no effect of the presence of low-frequency resistant mutants on virologic or immunologic response to therapy. The authors were unable to conclude that detection of low-frequency resistant mutants correlated with virologic or immunologic outcomes but the study was limited by small sample size, potential selection bias, and the limited number of mutants that could be detected by the sensitive assay employed.

Johnson and colleagues (Abstracts 61, 639) conducted a retrospective analysis of antiretroviral-naïve patients who participated in the treatment trials CNA 30021 and 30024 and received efavirenz and lamivudine with either abacavir or zidovudine. Of 316 patients, 95 had virologic failure (HIV-1 RNA lev-

el > 50 copies/mL) by 48 weeks, and 221 had suppressed viral loads (HIV-1 RNA level < 50 copies/mL) within 48 weeks. Using allele-specific PCR testing for K103N, Y181C, and M184V, 9 patients had low-frequency variants at baseline, 7 of whom (78%) had virologic failure. Five of 6 of the genotypes available at failure had the same mutations that were present at baseline. One patient who experienced failure within 2 months of treatment was found to have dual-class resistance (K103N, Y184V) at baseline. In logistic regression, presence of low-frequency variants at baseline was associated with virologic failure (OR, 11.0; 95% CI, 2.2–58.8, $P = .004$), but baseline viral load and baseline CD4+ count were not associated with virologic failure ($P = .43$ and $P = .30$, respectively). The authors concluded that there are clinical consequences to harboring low-frequency resistant variants and advocated for baseline sensitive testing, especially among antiretroviral-naïve individuals.

Global Perspectives on Antiretroviral Resistance

Schapiro (Abstract 59) presented an overview of the impact of HIV-1 subtype on drug resistance. A majority of drug development and resistance data have focused on HIV-1 subtype B, yet subtype B represents a minority of the infections worldwide (10%); other subtypes and circulating recombinant forms constitute the rest of infections worldwide.⁷ Furthermore, rates of non-subtype B infections are increasing in Europe and the United States (Abstracts 630, 648). Subtypes differ in genetic variability, which can lead to differences in response to antiretrovirals and development of drug resistance. Schapiro highlighted several examples of how this genetic variability before antiretroviral exposure can affect development of drug resistance mutations. Patients with subtype B who experience virologic failure in the context of nelfinavir treatment are more likely to develop the D30N PI resistance mutation than they are to develop the L90M mutation, whereas patients with subtype C are more likely to develop L90M.⁸

Subtypes B and C differ in consensus sequence at position 89 (89L and 89M, respectively). When the D30N mutation is inserted into a virus with 89M, there is no viral replication, whereas insertion of L90M allows for a replication capacity of 78.9%. This implies that 89M strains, and therefore a majority of subtype-C virus strains, do not replicate successfully with D30N mutation and as a result the L90M mutation is found more commonly than D30N among subtype C patients in whom nelfinavir-containing treatment is failing.⁹ Subtype genetic variability can also affect resistance through different codons that code for the same amino acid at key resistance positions. At position 106, both subtype B and C consensus sequences are V106, however, at the nucleotide level, subtype B sequence is GTA whereas subtype C is GTG. The NNRTI resistance mutation V106M is encoded by ATG. In order for subtype B V106 (GTA) to convert to V106M (ATG) it would require a 2-step conversion through GTG. In contrast, subtype C requires only a 1-step conversion: GTG to ATG. The hypothesis that the preferred pathway of resistance in subtype C at V106 is V106M is strengthened by the observation that clinically, among patients in whom efavirenz is failing the V106M resistance mutation is found more frequently in those with subtype C virus (24%) than those patients with subtype B virus (0.3%).¹⁰

Subtype C and Drug Resistance. Wallis and colleagues (Abstract 661) evaluated resistance patterns in 115 patients from 2 clinics in Johannesburg, South Africa, in whom antiretroviral therapy was failing, 94% of whom had subtype C virus. They found that resistance patterns were similar to those found in patients with subtype B virus with the exception of higher rates of V106M, K65R, G19A, and P225H mutations. The impact of these resistance mutations on second-line treatment is unclear and the authors suggest that these findings should be confirmed in larger controlled studies.

The nRTI resistance mutation K65R is relatively rare in subtype B and causes decreased replication capacity that can be augmented or decreased in

combination with other resistance mutations (Abstracts 591, 592). In areas where subtype C is prevalent, K65R is found at relatively higher rates¹¹ and is selected more rapidly in culture by subtype C than other subtypes.¹² Coutsinos and colleagues (Abstract 585) evaluated whether the effects of K65R mutation on reverse transcriptase in subtype C differ from its effects in subtype B. The authors found that subtype C recombinant virus with the K65R mutation had decreased replication capacity that is enhanced by M184V mutation and that M184V resensitizes K65R to tenofovir. These results are similar to what is found in subtype B.

Subtype G and Resistance. In Nigeria, subtype G and CRF 02-A/G are the predominant subtypes. Idigbe and colleagues (Abstract 641) evaluated the prevalence of resistance mutations among patients in Nigeria in whom a regimen of stavudine/lamivudine/nevirapine was failing. Of 125 patients, a majority were subtype G (43%) or CRF 02 (42%). Twenty-two (17.6%) were susceptible to all antiretrovirals, indicating likely non- or poor adherence as the cause of virologic failure, and 7 (5.6%) had resistance only to NNRTIs and 93 (74.4%) had resistance to NNRTI with TAMs or lamivudine resistance. The most common resistance mutations were M184V, Y181C, and K103N and there were no statistically significant differences in prevalence of resistance between treatment-experienced and treatment-naive patients nor among subtypes. There did, however, appear to be a higher frequency of TAMs (K70R and D67N) in patients with subtype G than in those CRF 02. Patterns of resistance in subtype G virus are similar to those described in subtype B infection but there was a higher prevalence of TAMs in subtype G than in CRF 02.

Schapiro (Abstract 59) concluded that although it is clear that genetic variability between subtypes impacts resistance, the clinical significance of this impact depends on the drug and the subtype. Thus far, subtype does not appear to influence antiretroviral therapy success or failure and should

not dictate a particular antiretroviral regimen. Resistance databases should continue to be expanded to include new information regarding resistance mutations in a variety of subtypes and algorithms should be updated accordingly. Furthermore, as new antiretroviral medications are developed, the potential effect of subtype variability on response to treatment should continue to be evaluated (Abstract 624).

Resistance in Resource-limited Settings. Marconi and colleagues (Abstract 94) presented rates of resistance after virologic failure among a cohort of patients in Durban, South Africa. One hundred and forty-one patients who had been on antiretroviral therapy for at least 24 weeks, were on their first regimen of potent antiretrovirals, or had history of prior mono- or dual-antiretroviral therapy, and who had virologic failure (defined as an HIV-1 RNA level >1000 copies/mL, failure to achieve at least a 1-log₁₀ copies/mL decrease in viral load after 4 weeks, or rebound after virologic suppression) were included in this cross-sectional study. Of these patients, 47.6% were on a regimen of stavudine/lamivudine plus either efavirenz (43.3%) or nevirapine (4.3%), and 31.2% were on a regimen of zidovudine/lamivudine plus either efavirenz (21.3%) or nevirapine (9.9%). In 71.6%, at least 1 significant resistance mutation emerged (63% nRTI, 66% NNRTI, 4% PI) and in 53.9%, dual-class mutations were present. Of patients with no prior history of antiretroviral therapy, those on a regimen of zidovudine/lamivudine/NNRTI had statistically significantly higher rates of mono- and dual-class resistance (approximately 85% and 69%, respectively) than patients on a stavudine/lamivudine/NNRTI regimen (approximately 70% and 53%, respectively). Patients who had a history of antiretroviral therapy had no statistically significant difference in presence of at least 1 resistance mutation but patients on a regimen of ritonavir-boosted lopinavir and 2 nRTIs had statistically significantly lower rates of dual-class resistance than patients on an NNRTI-based regimen (approximately 20% vs

80%, respectively). The most prevalent resistance mutation was M184V (54.6%) followed by K103N (43.3%), with K103N and M184V mutations appearing together at a rate of 33%. TAMs were present in 29% of patients with resistance with the TAM 2 pathway representing a majority of these mutations (17.7%). In multivariate analysis, presence of at least 1 resistance mutation and virologic failure were significantly associated with recent or history of opportunistic infection (OR, 3.10; 95% CI, 1.27-7.58) and history of HIV-1 RNA level below 300,000 copies/mL at enrollment (OR, 5.96; 95% CI, 1.08-32.8). Resistance was not associated with adherence but this may have been due to under-reporting of nonadherence, which was measured by self-report.

Chaix and colleagues (Abstract 646) presented data on a large cohort of patients in Côte d'Ivoire who received continuous antiretroviral therapy and were followed up for 6 months as part of a prerandomization phase for structured treatment interruption. The majority of patients were women (76%) and initiated a regimen of zidovudine/lamivudine/efavirenz (90%). At 6-month follow up, 10 patients had died, 1 was lost to follow up, and 15 had no viral load data available. One hundred seventeen patients (15%) had detectable HIV-1 RNA levels (above 300 copies/mL) of which samples from 112 were successfully amplified. Rates of resistance were 4.2% overall, 3.9% in patients on an NNRTI-based regimen, and 6.9% in patients on a PI-based regimen. Women who received zidovudine/nevirapine for PMTCT had high rates of resistance (20.4%) compared with women who received zidovudine/lamivudine/nevirapine (0%) or zidovudine alone (0%) for PMTCT. This cohort demonstrated a high rate of virologic success (85%) at 6 months, with low rates of virologic resistance (4.2%). However, the prevalence of low-frequency variants among patients in whom treatment failed but who did not have detectable resistance (70%) was not assessed.

In a cross-sectional analysis of HIV-1-infected pregnant women in the Gauteng Province of South Africa (Ab-

stract 640), 65 of 128 plasma samples from the year 2002 and 48 of 117 samples from the year 2004 were successfully amplified and evaluated for resistance mutations. No resistance mutations were identified among samples from 2002 and only 2 patients had resistance mutations (T69D, K70R) in samples obtained from 2004. Using the sensitive allele-specific real-time PCR assay, 1 additional sample was found to contain K103N. Prevalence of TDR was less than 5%. Although prevalence of resistance was low, it increased from 2002 to 2004. As availability of antiretroviral treatment increases, national surveys evaluating prevalence of drug resistance will be important in assessing trends in drug resistance.

In a cohort of 106 patients on antiretroviral therapy in Côte d'Ivoire (Abstract 645), patients were followed up for a median of 2 months to evaluate the correlation between baseline characteristics, including resistance, serious morbidity (WHO stage III-IV classification, hospitalization, or death), and immunologic failure (CD4+ count below 200 cells/ μ L). At baseline, 54% were on 2 nRTIs and a PI, 44% were on 2 nRTIs and an NNRTI, 58% had detectable viral loads (HIV-1 RNA level above 300 copies/mL), 20% had detectable viral loads without major resistance mutations, and 22% had detectable viral loads with at least 1 major resistance mutation. The most common mutations were M184V, D67N, M41L, K103N, and L90M. Detectable viral load with or without evidence of resistance was not predictive of serious morbidity, but presence of at least 1 resistance mutation was associated with immunologic failure (HR, 4.32; 95% CI, 1.38-13.57).

Sungkanuparph and colleagues (Abstract 663) evaluated rates and predictors of tenofovir resistance among tenofovir-naïve patients in whom a first-line regimen of stavudine/lamivudine/nevirapine was failing. Ninety-eight patients met inclusion criteria, which included history of undetectable viral load at 4 to 6 months after antiretroviral initiation and at least 2 subsequent HIV-1 RNA levels of above 1000 copies/mL. Ten patients were noted to have tenofovir resistance (6 with K65R and 4 with at

least 3 TAMs). All 10 patients with tenofovir resistance had concurrent NNRTI-resistance mutations. Factors associated with presence of tenofovir resistance included longer duration of antiretroviral therapy prior to detection of failure (OR, 1.12; 95% CI, 1.03-1.21) and higher level of HIV-1 RNA at time of failure (OR, 10.48; 95% CI, 1.77-62.13).

Resistance to New Antiretrovirals

Resistance to Tipranavir and Darunavir and Cross-resistance to PIs. Koh and colleagues (Abstract 606) conducted an *in vitro* analysis of emergence of darunavir resistance, comparing a wild-type strain of HIV-1 with polyclonal strains of HIV-1 obtained from 8 patients with known treatment failure of 9 to 11 antiretrovirals. They found that it was difficult to develop darunavir resistance in the wild-type strain but in the polyclonal multi-resistant strains, mutants with high levels of resistance to darunavir, saquinavir, amprenavir, indinavir, nelfinavir, ritonavir, and lopinavir emerged. The authors suggested that there is a significant barrier to development of darunavir resistance; however, in the setting of multi-resistant virus, high levels of resistance to darunavir can emerge.

In Abstract 607, investigators assessed darunavir cross-resistance to ritonavir-boosted amprenavir, tipranavir, and lopinavir by evaluating resistance profiles of 2682 patients with at least 1 major PI mutation. There was minimal evidence of cross-resistance between darunavir and atazanavir or tipranavir. There was more of a correlation of darunavir resistance with lopinavir resistance, and the tightest correlation was with amprenavir resistance. Patient-derived viral strains susceptible to darunavir were also susceptible to amprenavir, tipranavir, and lopinavir at rates of 100%, 89%, and 99.7%, respectively. Among patient strains with amprenavir resistance, 89% retained at least partial susceptibility to darunavir (39% remained completely susceptible to darunavir). In 84% and 88% of strains with tipranavir and lopinavir resistance, respectively, at least partial susceptibility to darunavir was

retained. Similarly, in Abstract 609, investigators found that patients with known virologic failure in the setting of amprenavir or evidence of amprenavir resistance retained good virologic response to darunavir at 48 weeks. Both studies found a correlation between genotypic evidence of resistance to darunavir and amprenavir, but this cross-resistance appeared to be overcome by the high potency of darunavir at the lower levels of amprenavir resistance. Furthermore, there did not appear to be easily definable cross-resistance of darunavir to other PIs.

Elston and colleagues (Abstract 602) evaluated development of cross-PI resistance in 62 patients who experienced virologic rebound while on a tipranavir-based regimen. A majority of failures were associated with the V82L/T mutation (47%) and 43% had other tipranavir resistance mutational patterns, typically L33F and 154V/A; 10% had no identified genotypic changes. Virus in 84% of all patients remained fully susceptible to darunavir and no patient had complete darunavir resistance. Virus remained susceptible to all other PIs with the possible exception of atazanavir. Patients with non-V82L/T tipranavir resistance mutations had increased susceptibility to all other PIs. Investigators from Tipranavir ANRS (Agence Nationale de Recherches sur le SIDA) study group (Abstract 612) identified mutations associated with loss of virologic response among PI-experienced patients on tipranavir-based regimens ($n = 143$). A tipranavir mutation score was developed based on virologic data from a multi-PI-experienced cohort and included amino acid positions 36 to 53, as well as 58, 69, and 89. In a multivariate analysis, previous enfuvirtide use (OR, 3.99; $P = .0015$) and a high tipranavir mutation score, implying higher numbers of tipranavir-associated resistance mutations, (OR, 6.8; $P < .001$) were associated with poorer virologic response, and an efavirenz background regimen (OR, 0.14; $P = .035$) was associated with better virologic response. Understanding the susceptibility and resistance patterns that emerge with tipranavir and darunavir treatment will

assist in optimizing the use of these agents in PI-experienced patients.

Resistance to Integrase Inhibitors. A series of in vitro studies were conducted with the investigational strand-transfer inhibitor elvitegravir to characterize resistance mutations to this integrase inhibitor (Abstract 627). Two major patterns of resistance to elvitegravir emerged: T66I and E92Q. T66I decreased sensitivity to elvitegravir 15-fold but had no effect on susceptibility to raltegravir. E92Q decreased susceptibility to both elvitegravir (30-fold) and raltegravir (6-fold). Minor mutations including F121Y and S147G were identified to have low-level resistance to elvitegravir and augment resistance conferred by T66I and E92Q. All 4 of these resistance mutations have been observed to cause resistance to other integrase inhibitors and have no effect on susceptibility to other classes of antiretrovirals including nRTIs, NNRTIs, and PIs.

Several studies evaluated the natural occurrence of integrase-inhibitor resistance in antiretroviral-naïve and treatment-experienced individuals. Integrase sequences from 2081 patients (1744 of whom were treatment-naïve) from the GenBank database were evaluated (Abstract 623) and included strains that were representative of a variety of subtypes including subtype C ($n = 504$), subtype A ($n = 288$), and subtype B ($n = 274$). Of 288 amino acid positions, 162 were polymorphic, all of which were in the non-catalytic region of integrase. There were low rates of conservative polymorphisms at extended active residues 141, 151, 155 and 156. Similarly, researchers from the Aaron Diamond AIDS Research Center (Abstract 625) found few polymorphisms associated with integrase-inhibitor resistance in vitro among 13 patients with multi-drug resistance virus and 103 recently infected, antiretroviral-naïve patients. Yerly and colleagues (Abstract 626) evaluated prevalence of integrase polymorphisms in 35 patients with subtype B and 54 patients with non-subtype B virus and found that polymorphisms associated with integrase-inhibitor resistance were present at similar levels in subtype B and non-subtype B isolates. The most

commonly occurring integrase-inhibitor-associated resistance mutations were V72I (17% of subtype B, 19% non-subtype B), V201I (11% subtype B, 17% non-subtype B), V165I (11% subtype B, 9% non-subtype B), and T206S (9% subtype B, 47% non-subtype B). The clinical significance of naturally occurring resistance to integrase inhibitors is an area of ongoing research.

Resistance to Entry Inhibitors. Baseline susceptibility to CCR5 blockers as HIV-1 entry inhibitors depends on the presence of a predominant CCR5-tropic virus population in an individual, and change in tropism may be one avenue for HIV-1 to escape from CCR5 blockade. Tropism was initially thought to be the major source of resistance to entry inhibitors. However, the major mechanism of resistance to CCR5 blockers, such as maraviroc, appears to result from mutations that permit HIV-1 to utilize the CCR5 coreceptor despite the presence of bound inhibitor (Abstract 108). The following 2 abstracts conducted clinical and in vitro studies to evaluate factors associated with tropism switch in HIV-1 strains.

The rate and predictors of tropism switch among chronically infected patients with known antiretroviral drug resistance was evaluated among patients in the Study on the Consequences of the Protease Inhibitor Era (SCOPE) cohort (Abstract 619). Sixty-six patients met inclusion criteria, which included baseline plasma HIV-1 RNA level above 1000 copies/mL, presence of at least 1 major or 1 minor genotypic resistance mutation, and use of stable antiretroviral regimen for 120 days or more before baseline. Patients were followed up until regimen change. At baseline, 52, 22, and 2 patients had CCR5-, dual and mixed-, and CXCR4-tropic virus, respectively. Risk of progression from CCR5 to dual and mixed tropism at 1 year was 12% (95% CI, 6-26%) and in multivariate analysis, presence of CCR5 $\Delta 32$ heterozygosity was independently predictive of switch ($P = .024$). Risk of switch from mixed and dual to pure CXCR4 and to CCR5 was 8% (95% CI, 1-43%) and 11% (95% CI, 3-37%), respectively. Neither of the

2 CXCR4-tropic viruses identified at baseline had observed tropism switch. Of populations noted to have a tropism switch, 30% showed small changes in CXCR4 at entry as measured by reductions in relative light units, implying that the clinical significance of switch in these populations may be limited. This study implies that deferring a change in antiretroviral treatment among patients with known resistance may carry a small risk of losing CCR5 tropism (approximately 10%).

Moncunill and colleagues (Abstract 618) conducted *in vitro* studies of CCR5 coreceptor switches in the presence and absence of CCR5, CXCR4, and reverse transcriptase inhibitors (RTIs). They found that in the absence of drug pressure, 3 of 6 strains switched from CCR5 to CXCR4 and demonstrated increased rates of replication. In the presence of CCR5 antagonists, CXCR4-using virus emerged more quickly in the presence of TAK-799 but was prevented by plerixafor (AMD3100). These studies indicate that cell culture models can be useful in predicting the propensity of clinical isolates to develop entry inhibitor resistance in the setting of antiretroviral drug pressure.

Antiretroviral Resistance Mutations: Interactions and Effects on Fitness

Resistance to Nucleoside Reverse Transcriptase Inhibitors. Sluis-Cremer presented an overview of mechanisms of resistance to nRTIs and interactions among RTI resistance mutations (Abstract 58). HIV-1 reverse transcriptase is essential for transcribing an RNA template into DNA. The nRTIs are nucleoside or nucleotide analogues that compete with the naturally occurring deoxynucleotide triphosphate (dNTP) and cause chain termination that prevents formation of HIV DNA. Previous studies have shown that resistance to nRTI occurs due to preferential incorporation dNTP over the nRTI (a process known as discrimination) or increased excision of the nRTI. Kinetic data have shown that with the K65R, K70E, L74V, M184V, and Q151M mutations, resistance occurs through discrimination, whereas TAM-mediated resistance oc-

curs through facilitated excision of the nRTI. These mechanisms of resistance appear to be antagonistic; there is evidence that when M184V, L74V, or K70E occur in conjunction with TAMs, the virus or enzyme is less susceptible to zidovudine. K65R and TAMs (of the TAM 67 pathway) appear to have bidirectional antagonism: clinical isolates with both mutations are infrequently observed. The presence of K65R with TAM 67 decreases the resistance to zidovudine in the presence TAM 67 alone from 54-fold to 1.5-fold, and TAM 67 with K65R decreases the resistance to tenofovir in the presence of K65R alone from 4.2-fold to 2.4-fold.

Sluis-Cremer also provided evidence that antagonism among resistance mutations affects virologic response. In the ESS30009 study¹⁵, at 12 weeks 49% (50 of 102) of patients randomized to tenofovir/abacavir/lamivudine were virologic nonresponders. Of these virologic nonresponders, 98% had M184V/I, either in combination with (44%) or without (53.5%) K65R. This relatively high rate of virologic nonresponse could be the result of a low genetic barrier to emergence of M184V and K65R and the fact that K65R confers resistance to all of the medications in the regimen.¹⁴ This is in contrast to the A5095 study¹⁵ in which patients randomized to receive zidovudine/abacavir/lamivudine had a rate of virologic nonresponse of only 21% (82 of 382). Of these nonresponders, 70% had evidence of resistance: 51% (29 of 57) with M184V alone, 14% (8 of 57) with TAMs and M184V in combination, and 3.5% (2 of 57) with TAMs alone. Sluis-Cremer hypothesized that the relatively low rate of virologic nonresponse could be attributed to the higher genetic barrier to TAMs and M184V, as well as the antagonism between TAMs and M184V.

K65R. The K65R mutation is associated with decreased susceptibility to nRTIs and decreased replication capacity. In a database analysis of patients in whom tenofovir therapy failed (Abstract 591), K65R was often accompanied by A62V and S68G mutations. Site-directed mutagenesis was conducted to create

K65R, K65R/A62V, K65R/S68G, and K65R/A62V/S68G mutants, and their fitnesses were compared with wild-type virus in the presence and absence of tenofovir. The results suggested that A62V and S68G act as partial compensatory mutations for K65R by increasing replication capacity. In the absence of tenofovir, wild-type virus was more fit than the K65R/A62V/S68G mutant, but in the presence of greater than 7 μ M of tenofovir, the triple mutant was more fit. Incorporation kinetics showed that the K65R mutants with A62V/S68G exhibited more efficient incorporation of dATP and dGTP than mutants with K65R alone.

In contrast to A62V and S68G, the K70E mutation, which is associated with patients in whom tenofovir-containing regimens are failing, is rarely found in conjunction with K65R (Abstract 584). Site-directed mutagenesis has shown very poor replication capacity among K70E/K65R double mutants, suggesting antagonism between these mutations.¹⁶ Using molecular dynamic simulations, Kagan and colleagues (Abstract 592) evaluated whether there was a structural basis to this antagonism. In wild-type virus, the K65 was found to stabilize the triphosphate moiety of the dNTP ligand but with K65R mutation the stabilization was lost. The K70 in wild type appears to compensate for the loss of stabilization conferred by the K65R mutant. The double mutant K65R + K70E has no compensation for this loss of stabilization, which leads to a more severe defect, thus providing a structural basis for the antagonism between the K65R and K70E mutants. Molecular dynamic simulation may be a useful tool to evaluate other antagonistic mutation interactions.

Resistance to Enfuvirtide. Enfuvirtide blocks fusion of HIV with CD4 through competitive interactions with the HR1 and HR2 helices of the gp41 transmembrane protein. Genotypic changes in HR1 and HR2 have been associated with resistance to enfuvirtide. Resistance mutations in the HR1 (N43D) and HR2 (E137K) sequences among 5 enfuvirtide-experienced patients were

identified, cloned, and evaluated for their effect on fitness and response to enfuvirtide through in vitro assays of viral envelope fusogenicity and infectivity (Abstract 620). N43D single mutants and N43D/137K double mutants had decreased susceptibility to enfuvirtide compared with wild type at magnitudes of 28-fold and 32-fold, respectively. However, the N43D single mutant had decreased fitness compared with wild type by 92%, and the N43D/E137K double mutants had no statistically significant difference in fitness compared with wild type. The E137K single mutant conferred no statistically significant difference in sensitivity to enfuvirtide or in fitness compared with wild type. The N43D mutation appeared to decrease susceptibility to enfuvirtide, but at a substantial cost to fitness, for which the E137K mutation can compensate. An analysis of previously published data regarding prevalence of resistance mutations to enfuvirtide supported this hypothesis: the N43D mutation occurred at higher rates among patients with E137K/Q mutations than those without E137K/Q mutations.

Resistance to Protease Inhibitors.

Previous studies have shown that amino acid inserts near the cleavage and noncleavage sites in Gag compensate for decreased viral fitness secondary to PI-resistance associated mutations. Aoki and colleagues (Abstract 601) evaluated the effect of a 7 amino acid insert (TTNTRNS) near the p17/p24 cleavage site in a heavily drug-experienced, HIV-infected patient. Samples were obtained throughout the course of treatment that included a regimen of zidovudine/lamivudine/nelfinavir followed by stavudine/ritonavir-boosted saquinavir and then stavudine/ritonavir-boosted saquinavir/abacavir. Seventy percent of clones from the patient contained the TTNTRNS insert and were found early in the course of antiretroviral treatment. The presence of the insert had no effect on the propensity to acquire nelfinavir mutations. Introduction of the insert to wild-type virus resulted in decreased fitness. However, clones with saquina-

vir-, indinavir-, nelfinavir-, or amprenavir-associated mutations containing the insert had increased fitness. In this study, presence of TTNTRNS insert near the p17/p24 cleavage sites improved the fitness of otherwise replication-compromised PI-resistant mutants.

Hypersusceptibility to Non-nucleoside Reverse Transcriptase Inhibitors.

The nRTI induction of NNRTI hypersusceptibility and accompanying influences on virologic response have been previously described but the mechanism of this mutational interaction has remained unclear. Clark and colleagues (Abstract 598) evaluated 3 nRTI mutations (I181I, 208Y, and 215Y) that are strongly associated with NNRTI hypersusceptibility and their effects in vitro on fitness and reverse transcriptase activity. Replication capacity varied depending on the combination of mutations present: 208Y/215Y, I181I/208Y/215Y, and I181I/215Y mutants had replication capacities of 40%, 35%, and equal to wild type, respectively. The effect of mutation combination on reverse transcriptase activity and polymerase activity of reverse transcriptase was similar to its effect on replication capacity: 208Y/215Y, I181I/208Y/215Y, and I181I/215Y exhibited 47%, 30%, and equal to reverse transcriptase activity compared with wild type, respectively. It was hypothesized that hypersusceptibility to NNRTIs conferred by 208Y/215Y and I181I/208Y/215Y mutants may be related to altered gag-pol processing and the mechanism of hypersusceptibility conferred by I181I/215Y double mutants is distinct and remains to be determined.

Fading of Resistance Mutations with Treatment Interruption.

Ceccherini-Silberstein and colleagues (Abstract 587) evaluated the evolution of resistance mutations among 138 patients experiencing virologic failure who underwent treatment interruption for at least 1 month. Genotypes were available at the time of virologic failure and at least once during treatment interruption. Disappearance of resistance mutations correlated with known effects of resistance on fitness. M184V,

Y115F, and K65R had completely disappeared within 4 months of treatment interruption and were associated with the fastest viral load increase, which is consistent with their detrimental effects on fitness. In contrast, TAMs, L74V, E44D, and H208Y progressively declined during treatment interruption but were still present in more than 10% of individuals 9 months post-treatment interruption. E44D, V181I, E203K, and H208Y were all associated with maintenance of TAM 1 pathway mutations and K20R and D218E were associated with persistence of TAM 2 pathway mutations. The dynamics of resistance mutation evolution in the absence of drug pressure appears to be related to the relative fitness of the virus conferred by resistance mutations and resistance mutation combinations. Prevalence of low-frequency resistance variants, however, was not assessed.

Charpentier and colleagues (Abstract 622) evaluated the disappearance of mutations associated with resistance to enfuvirtide after treatment discontinuation. Bulk sequencing was used to detect resistance mutations in 7 patients who had immunovirologic failure while on enfuvirtide at baseline, during treatment, and after treatment. Molecular cloning was used to detect low-frequency resistance variants. Median medication duration was 6 to 4 months. Of 7 patients, 3 had complete reversion to wild type, 2 had persistence of enfuvirtide-resistant virus as a minority population, and 2 had persistence of resistance as a majority population. A high proportion of patients in whom resistance persisted were those who received enfuvirtide for long periods of time (> 6 months). There was no association between enfuvirtide resistance mutations and the kinetics of mutation disappearance. Further investigation is required to determine the clinical significance of persistent enfuvirtide resistance.

Mutations in the Connection and RNase H Domains of Reverse Transcriptase

The HIV-1 reverse transcriptase is composed of 3 domains of the p66 subunit

(polymerase, connection, and RNase H) and 2 domains of the p51 subunit (polymerase and connection). Most resistance assays sequence only the polymerase domain and do not evaluate for resistance mutations in the connection and RNase H domains. Several studies at this year's conference presented evidence that mutations in these infrequently analyzed domains may be clinically significant.

Mutations in the RNase H Domain.

The function of RNase H is to cleave the RNA moiety of RNA and DNA hybrids during reverse transcription. Recent studies have shown that HIV-1 reverse transcriptase mutations in the RNase H domain can increase nRTI resistance, presumably by decreasing RNase H activity, allowing more time for excision of the incorporated nRTI-monophosphate.¹⁷

To evaluate mutations in reverse transcriptase outside of the catalytic domain, selections of zidovudine-resistant HIV-1 were made in vivo and the entire coding region of reverse transcriptase was sequenced (Abstract 90). Sequencing identified the A371V mutation (located in the connection domain) and Q509L mutation (located in the RNase H domain). Using site-directed mutagenesis, A371V and Q509L mutants were found to be 1.7-times more resistant to zidovudine than wild type, but in conjunction with TAMs (D67N and K70R), resistance increased to 39-fold that of wild type (compared with a 4.6-fold increase with TAMs alone). The mechanism of augmented resistance by A371V and Q509L appeared to be a decrease in RNase H cleavage activity, which may lead to facilitated zidovudine excision in the presence of TAMs. RNase H is also being evaluated as a novel target for antiretroviral therapy (Abstract 89), further emphasizing the need to evaluate the effects of RNase H activity on resistance and fitness.

Mutations in the Connection Domain: N348I. Abstracts 593 and 594 described the resistance mutation N348I, which is found in the connection domain and confers resistance to

NNRTIs and nRTIs. Hachiya and colleagues (Abstract 593) identified 2 clinical isolates that were phenotypically highly resistant to nevirapine and delavirdine despite a lack of NNRTI-associated resistance mutations. Sequencing of the entire reverse transcriptase domain of these isolates identified the N348I. Compared with wild type, N348I mutants conferred increased resistance to zidovudine (8.6-fold), didanosine (5-fold), nevirapine (22-fold), and delavirdine (4.3-fold).

Yap and colleagues (Abstract 594) performed genotyping on 1377 treatment-naïve and treatment-experienced patients and identified N348I as the ninth most prevalent mutation, occurring 11.3-times more frequently in treatment-experienced patients than in treatment-naïve patients. N348I appeared relatively early in virologic failure, before the appearance of TAMs and about the same time as NNRTI-resistance mutations, and was associated with zidovudine and combination zidovudine/nevirapine treatment. N348I decreased susceptibility to zidovudine between 2-fold alone and 4-fold in combination with TAMs, did not antagonize M184V resistance to zidovudine, conferred resistance to efavirenz and nevirapine as a single mutant, and augmented K103N-related resistance to efavirenz and nevirapine. Molecular dynamics simulation suggested that the N348I mutation inhibits movement of the thumb region of the polymerase domain, thereby allowing more time for zidovudine excision. These studies indicate that this novel N348I has clinical relevance and suggest that genotypic and phenotypic analysis of the entire reverse transcriptase should be conducted to identify the prevalence of other clinically-significant resistance mutations outside of the polymerase domain.

Predictors of Immunologic, Virologic, and Clinical Outcomes

From the first days of the Multicenter AIDS Cohort Study (MACS), investigators have gained insights by following up groups of HIV-infected individuals

over time. The following section is a selected review of predictors of clinical outcomes from cohort studies at this year's conference.

Sabin and colleagues (Abstract 528) presented an analysis from the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE), a collaboration of 33 cohorts from 30 European countries contributing data on 827 children under 17 years of age and 49,094 adults. Patients eligible for inclusion in this study ($n = 49,921$) were antiretroviral therapy naïve, aged 6 years or older, had initiated antiretroviral therapy between 1998 and 2006, and had more than 1 CD4+ cell count and plasma HIV-1 RNA measurement taken pre-antiretroviral initiation and during follow up. Baseline characteristics included median age of 37 years, 29% female, CD4+ count of 210 cells/ μL , and plasma HIV-1 RNA level of 4.9 \log_{10} copies/mL. Thirty-eight percent had initiated an NNRTI-based regimen and 28% had initiated non-ritonavir-boosted PI regimens.

Age was found to be a predictor of immunologic, virologic, and clinical outcome. Immunologic responses to therapy in the cohort were appropriate, with 59.2% achieving a confirmed CD4+ cell response by 1 year, but the probability of an immunologic response was higher in younger individuals (particularly those under 12 years of age) and reduced in those over 60 years of age. In the first year of therapy, 53.7% of patients achieved a virologic response to antiretroviral treatment, but the probability of response was lower in those aged 6 to 17 years, and higher in those aged 50 years and older than those aged 30 to 39 years. Older individuals were more likely to develop an AIDS-defining event or death in the first year on antiretrovirals, with adjusted HRs of 1.19 (95% CI, 1.05-1.34) and 1.34 (95% CI, 1.19-1.51) for those aged 55 to 59 years and over 60 years.

Braithwaite and colleagues (Abstract 520) examined the relationship between antiretroviral regimen, adherence, and virologic response in a cohort of 6394 patients initiating antiretroviral therapy within the Veterans

Affairs Healthcare System. Initiation of treatment with NNRTI-based regimens was compared with initiation of ritonavir-boosted PI-based regimens. Adherence, estimated from pharmacy refill data, was statistically significantly greater with efavirenz-based regimens (67%) and nevirapine-based regimens (65%) than with boosted (59%) or unboosted (61%) PI-based regimens ($P < .001$). In multivariate analyses, plasma HIV-1 RNA suppression was inferior in nevirapine-based regimens (OR, 0.60), boosted PI- (OR, 0.57), and unboosted PI-based regimens (OR 0.48) compared with efavirenz (all, $P < .001$).

Using data from the Swiss HIV Cohort Study, predictors for long-term CD4+ count increases in 2860 patients initiating first-line antiretroviral therapy were evaluated (Abstract 518). Sixty-three percent achieved virologic suppression, and median CD4+ count increases were 87, 52, and 19 cells/ μ L in the 3 time periods examined: 1, 2 to 3, and 4 to 5 years after plasma HIV-1 RNA suppression, respectively. In multivariate modeling, median CD4+ count increase was statistically significantly higher for patients with female sex ($P < .001$), lower age ($P < .001$), higher plasma HIV-1 RNA at start of antiretroviral therapy ($P = .002$), and CD4+ count of below 650 cells/ μ L at start of the period ($P = .010$).

Another evaluation of the Swiss HIV Cohort Study determined the predictive value of longitudinal self-reported adherence assessment for virologic rebound, defined as 2 consecutive plasma HIV-1 RNA measurements above $2.7 \log_{10}$ copies/mL (Abstract 523). Patients were included in the analysis if they were on antiretroviral therapy, over 16 years of age, had plasma HIV-1 RNA below $1.7 \log_{10}$ copies/mL over the previous 3 months, and had completed adherence questionnaires before June 1, 2006. Among the 2638 subjects who met inclusion criteria, the median follow up was 2.5 years. Patients reported missing 1 or more doses at 25% of visits, and missing more than 2 doses at 9.9% of visits. A total of 97 patients (3.7%) experienced virologic rebound. In an unadjusted analysis, there was no difference in rates of vi-

rologic failure between patients who reported perfect adherence and those who reported missing 1 dose. HRs for 2 missed doses and more than 2 missed doses were 1.89 (95% CI, 1.24-2.88) and 3.88 (95% CI, 2.74-5.48), respectively. In a multivariate analysis, nonadherence, defined as a self-report of missing 2 or more doses of medication in the previous 28 days, was associated with an increased risk of virologic rebound (HR, 2.82; 95% CI, 1.76-4.50). Other risk factors for virologic rebound were having had more than 5 previous antiretroviral regimens (HR, 2.75; 95% CI, 1.65-4.61) and comedication for cardiovascular problems, opportunistic infections, or hepatitis C virus infection (HR, 2.42; 95% CI, 1.54-3.82).

Gibb and colleagues (Abstract 701) combined data from 11 studies of HIV-1-infected children in resource-limited settings to examine factors predicting mortality for these children. Ten African studies and 1 Brazilian study of untreated children older than 12 months were used to form a retrospective cohort of 2510 children, 3769 person-years of observation, and 357 deaths. The majority (81%) of the follow up occurred after the initiation of co-trimoxazole therapy. The first available data points were used as baselines, and the investigators found a median age of 4.0 years, CD4+ cell percentage of 15, and weight-for-age z-score of -1.9. Predictors of mortality were CD4+ cell percentage, CD4+ count, weight-for-age, and hemoglobin level. Children with weight-for-age z-scores lower than -3 and hemoglobin level of below 8 mg/dL had a mortality rate of 55.2 per 100 person-years compared with 1.4 per 100 person-years when weight-for-age z-score was 1 or higher and hemoglobin level was 10 mg/dL or higher. This trend was seen even in children who had a baseline CD4+ cell percentage of more than 15. This report highlights the need for consideration of weight-for-age and hemoglobin level in decisions regarding antiretroviral therapy initiation in resource-limited settings, as is recommended in the most recent WHO antiretroviral treatment guidelines.

Clinical Outcomes Associated with Resistance

Phillips and colleagues (Abstract 532) presented data from the UK Collaborative HIV Cohort Study (UK CHIC) on the cumulative risk of extensive triple-class failure. They defined failure of a drug as plasma HIV-1 RNA above $2.6 \log_{10}$ copies/mL after more than 4 months of continuous use of that drug. Extensive failure of the nRTI class was failure of at least 1 drug from each of the following sub-classes: zidovudine and stavudine; lamivudine and emtricitabine; and didanosine, tenofovir, and abacavir. Extensive failure of NNRTIs was determined by failure of either nevirapine or efavirenz, and extensive failure of PIs involved virologic failure of at least 1 ritonavir-boosted PI. Extensive triple-class failure was defined as failure of all 3 classes. Of 10,603 patients evaluated, 25% were female, and median age, CD4+ count, and viral load were 36 years, 185 cells/ μ L, and $4.96 \log_{10}$ copies/mL, respectively. During 38,190 person-years of observation, 169 patients developed extensive triple-class failure, 70% of whom had at least 1 prior measurement of plasma HIV-1 RNA below $1.7 \log_{10}$ copies/mL. Of 169 patients, 95 (56%) subsequently had at least 1 plasma HIV-1 RNA measurement below $1.7 \log_{10}$ copies/mL. A baseline CD4+ count of below 200 cells/ μ L carried a HR of 2.2 for extensive triple-class failure ($P < .0001$), and for those with a baseline CD4+ count above 200 cells/ μ L, the cumulative risk of extensive triple-class failure was 4% (95% CI, 2-6).

The correlation between resistance and clinical outcomes was analyzed among 1929 antiretroviral-naive patients in the Swiss HIV Cohort Study who initiated treatment with at least 2 nRTIs plus an NNRTI or ritonavir-boosted PI ($n = 518$) from January 1999 to December 2005 (Abstract 667). Sixty-nine of 805 (8.6%) patients in the NNRTI group and 24 of 518 (4.6%) in the PI/ritonavir group experienced virologic failure, which was defined as viral load of 500 copies/mL or higher after at least 180 days on continuous treatment. Although discontinuation of

antiretroviral therapy due to virologic failure was not statistically significantly different between the groups, discontinuation due to an adverse event was more common among patients on an NNRTI than among patients on PI/ritonavir ($n = 189$ vs $n = 122$, respectively, log rank $P = .0241$). Among patients for whom genotypic resistance testing was available ($n = 1323$), patients on a PI/ritonavir regimen had higher rates of susceptibility to lamivudine/emtricitabine (75% vs 42.1%, $P = .026$) and to a third antiretroviral drug (90% vs 52.6%, $P < .001$) than patients on an NNRTI-based regimen. There was no statistically significant difference in susceptibility to nRTIs. Although rates of virologic failure were not different between PI/ritonavir-based regimens and NNRTI-based regimens, rates of adverse events and resistance were higher among patients initiated on an NNRTI-based regimen.

Predictors of mortality were evaluated among patients enrolled the Danish HIV Cohort study who experienced triple-class virologic failure from 1995 to 2004. Triple-class failure was defined as viral load above 1000 copies/mL for a total of 120 days while on antiretroviral treatment, and median time of follow up after triple-class failure was 4.3 years. One hundred and seventy patients experienced triple-class failure, 133 of whom received resistance testing. The median number of resistance mutations was 8 and 61% (81 of 133) had resistance to 3 major classes. Mortality from time of triple-class failure was 70 (95% CI, 54-92) per 1000 person-years in patients who experienced triple-class failure compared with 29 (95% CI, 26-32) per 1000 person-years from time of antiretroviral initiation in all patients in the cohort. In multivariate analysis, mortality rate ratio (MRR) was associated with presence of 9 or more resistance mutations (MRR, 2.3; 95% CI, 1.1-4.8), presence of T215Y (MRR, 3.4; 95% CI, 1.6-6.66), G190A/S (MRR, 3.2; 95% CI, 1.6-6.6), or V82F/A/T/S (MRR, 2.5; 95% CI, 1.2-5.3). After adjusting for latest CD4+ count, only presence of T215Y and latest CD4+ count remained associated with mortality. The authors concluded

that a majority of resistance mutations among patients with triple-class failure accumulated during suboptimal treatment in the 1990s and that perhaps T215Y is a marker of earlier development of immunodeficiency. With the availability of newer drug classes, it remains to be seen if multi-class failure to RTIs and PIs will remain associated with mortality.

The relationship between mortality and use of resistance testing was analyzed using data from the HIV Outpatient Study (HOPS) (Abstract 660). Of patients enrolled in the cohort since January 1999, 3202 were evaluated and had median follow up of 3.3 years. Resistance testing was performed in 1110 of these patients. Patients who were white, had private insurance, had a lower CD4+ count, and whose risk behavior was MSM were more likely to have had resistance testing performed. Among patients who had received potent antiretroviral therapy ($n = 2107$), receiving a resistance test and private health insurance were associated with decreased mortality even after adjusting for stage of HIV disease, demographics, age, and year (HR, 0.60 and 0.63, respectively). Among patients who were antiretroviral naive with CD4+ counts below 200 cells/ μ L ($n = 257$), resistance testing before initiation of antiretroviral therapy was also protective (HR, 0.22, although 95% CI approaches 1.0). Although limited by the retrospective nature of the study design, this analysis provides evidence that antiretroviral therapy guided by resistance testing is associated with a substantial clinical benefit.

Host-factor Influence on Response to Therapy

The human leukocyte antigen (HLA) variants Bw4 and Bw6 help determine HLA interactions with natural killer cells, and Bw4 has been associated with enhanced control of HIV infection. Rauch and colleagues (Abstract 141) combined data from the Swiss HIV Cohort Study and the Western Australia HIV Cohort Study to examine the effects of Bw4 on immunologic and virologic responses to antiretroviral ther-

apy. Data from 161 Bw4+ adult, white men initiating antiretroviral treatment between 1997 and 2002 were analyzed. In the Australian cohort, baseline mean age of Bw4+ individuals was 4 years older than Bw4- individuals, and in the Swiss cohort, mean CD4+ count of Bw4+ individuals was 90 cells/ μ L lower than Bw4- individuals. Baseline plasma HIV-1 RNA, follow-up time, initial antiretroviral regimen, and mode of infection did not differ significantly between Bw4+ and Bw4- individuals in either cohort. CD4+ percentage and absolute CD4+ counts were consistently lower in Bw4+ carriers than in the remainder of the cohort from 1 to 5 years on antiretrovirals. CD4+ counts were approximately 85 cells/ μ L lower in Bw4+ than Bw4- individuals in the Swiss cohort and 55 cells/ μ L lower in the Australian cohort ($P = .005$ and $P = .01$, respectively). These differences were more profound in patients carrying the variant Bw4-80T, and remained statistically significant after adjusting for virologic response rates.

Rosignoli and colleagues (Abstract 451) presented data on the effect of antiretroviral therapy on expression of CD279, also known as programmed death 1 (PD-1), and its ligand (PD-L1), which has been implicated in promoting and regulating energy of HIV-1-specific CD8+ cells. They examined PD-1 and PD-L1 activity in 22 HIV-1-infected individuals on antiretroviral therapy who had plasma HIV-1 RNA below 1.7 log₁₀ copies/mL and a median CD4+ count of 547 cells/ μ L, and compared it with levels in 10 uninfected controls. There were no statistically significant differences in levels of PD-1, even after stratification by antiretroviral treatment regimen. Previous studies have shown higher levels of PD-1 in untreated viremic patients, so this may represent a normalization of PD-1 levels with antiretroviral therapy. The mean fluorescence intensity of PD-L1 on T cells was higher in HIV-1-infected individuals regardless of their antiretroviral therapy status. Evidence of PD-1 activity could be a signature of the persistent anergic state.

Liptrott and colleagues (Abstract 452) also explored the relationship

between PD-1 and response to anti-retroviral treatment, but they focused on PD-1.3, an allele of the PD-1 gene that has been shown to alter the regulation of PD-1 gene expression. PD-1.3 genotyping and an assay for CCR5 Δ 32 were conducted on samples from 77 antiretroviral-naïve patients initiating efavirenz-based regimens. Median CD4+ count at baseline was 202 cells/ μ L and median plasma HIV-1 RNA level was 4.9 log₁₀ copies/mL. Fifteen heterozygotes and 1 homozygote for PD-1.3 and 7 heterozygotes for CCR5 Δ 32 were identified. Baseline median CD4+ counts were statistically significantly lower in patients with PD-1.3 alleles (121 cells/ μ L, range 5-335) than patients with wild type (187 cells/ μ L; range 10-760; $P = .02$ for the difference between the 2 CD4+ counts). CD4+ counts were also significantly lower in individuals with the PD-1.3 allele at 2, 4, 6, and 8 months after initiation of antiretroviral therapy. There were no significant differences in virologic response between the 2 groups, and CCR5 Δ 32 did not have an appreciable effect on response to therapy. The results were limited by small sample size, but suggest that further investigations are needed.

Selected Pharmacokinetic Presentations

Antiretroviral Drug Interactions

Waters and colleagues (Abstract 557) presented data on the interaction of abacavir with atazanavir/ritonavir and lopinavir/ritonavir in HIV-infected patients. The pharmacokinetics of abacavir before and after adding 2 weeks of either atazanavir/ritonavir or lopinavir/ritonavir and the pharmacokinetics of atazanavir/ritonavir or lopinavir/ritonavir before and after adding abacavir were evaluated. Atazanavir/ritonavir and lopinavir/ritonavir levels were not affected by the addition of abacavir, however, the AUC (area under the concentration curve) of abacavir was decreased by 17% after the addition of atazanavir/ritonavir and by 32% after the addition of lopinavir/ritonavir. The

mechanism of this interaction and the clinical significance are not clear.

Kakuda and colleagues (Abstract 560) presented data from TMC125-C223, a phase II study of etravirine (an investigational NNRTI) in treatment-experienced subjects. The pharmacokinetic parameters of etravirine were decreased by coadministration of either a PI or tenofovir. Etravirine trough concentrations, AUC, and de novo enfuvirtide use were associated with improved virologic response. The association between pharmacokinetic parameters and virologic response appeared to be relevant in the lower but not the higher dose group.

Bertz and colleagues presented pharmacokinetic and pharmacodynamic data from BMS 424-089, a study comparing ritonavir-boosted atazanavir to unboosted atazanavir with 2 nRTIs in treatment-naïve subjects (Abstract 565). As expected, trough concentration of atazanavir was lower in subjects receiving unboosted atazanavir than subjects receiving boosted atazanavir (125 ng/mL and 663 ng/mL, respectively). The trough concentration correlated with probability of having a plasma HIV-1 RNA level below 50 copies/mL at week 48 and higher bilirubin levels, but did not correlate with changes in lipid parameters. The authors suggested that the relatively adverse lipid effects seen with boosted atazanavir versus unboosted atazanavir were due to ritonavir administration, not due to higher atazanavir levels per se.

Tebas and colleagues (Abstract 572) presented data on the pharmacokinetics of enfuvirtide in patients with severe renal impairment (defined as calculated creatinine clearance of 11 to 35 mL/minute) and patients on hemodialysis. They found that the AUC of enfuvirtide was higher in patients with renal disease (80.3 μ g·h/mL in patients with severe renal impairment and 71.1 μ g·h/mL in patients with end-stage renal disease [ESRD]) than in controls with normal renal function (49.6 μ g·h/mL). Despite the higher exposure in patients with renal disease, all patients tolerated enfuvirtide well and no safety concerns were identified, therefore no dose adjustment was recommended.

Interactions Between Antiretroviral and Non-antiretroviral Medications

Hoody and colleagues (Abstract 564) presented data on the interaction of lopinavir/ritonavir and rosuvastatin, an HMG Co-A reductase inhibitor. A drug-drug interaction was not expected as rosuvastatin is not a substrate for CYP3A4. However, investigators found that rosuvastatin AUC was increased 2.1 fold and maximum concentration (C_{max}) by 4.7 fold. The authors suggested that a dose separation strategy should be tested to overcome this interaction.

Agarwala and colleagues (Abstract 568) investigated several strategies to coadminister famotidine with atazanavir/ritonavir and tenofovir through pharmacokinetic studies with HIV-seronegative volunteers. Four different famotidine dosing strategies led to modest reductions in the minimum concentration (C_{min}) of atazanavir: 20 mg orally twice daily (morning dose of famotidine and atazanavir/ritonavir coadministered) led to a 19% reduction, 20 mg orally twice daily (atazanavir/ritonavir and famotidine separated by 2 hours) led to an 18% reduction, 40 mg orally once daily (separated by 12 hours from atazanavir/ritonavir) led to a 23% reduction, and 40 mg orally twice daily led to a 28% reduction.

Rifampin is known to decrease levels of PIs, precluding their coadministration. Acosta and colleagues (Abstract 575) presented data from ACTG A5213, which studied higher doses of atazanavir without ritonavir to overcome this interaction. Regimens studied included atazanavir 300 mg twice daily, atazanavir 400 mg twice daily, and atazanavir 600 mg twice daily. Even at the highest dose, the trough concentration of atazanavir (55 ng/mL) was statistically significantly lower than that of historic controls receiving 400 mg daily (159 ng/mL). Coadministration of these drugs is therefore not recommended.

German and colleagues (Abstract 577) presented data on the interaction of efavirenz and a leading antimalarial treatment, artesunate and amodiaquine. The study was stopped early af-

Table 4: Key Findings and Potential Clinical Implications

Clinical Trials of Antiretroviral Agents	
Summary	Potential Clinical Implications
Treatment of antiretroviral-experienced patients (Abstracts 104aLB, 104bLB, 105aLB, 105bLB)	
2 studies of maraviroc and 2 studies of raltegravir achieved excellent virologic suppression in highly treatment-experienced subjects when using these new agents. The best responses occurred when there were 1 or more active drugs in the optimized background regimen. These drugs appeared safe and well tolerated.	Achieving complete virologic suppression (plasma HIV-1 RNA <50 copies/mL) is a realistic goal for all patients initiating or changing antiretroviral therapy. New agents, especially those in new drug classes, are optimally given when there is at least 1 other agent to which the patient is sensitive.
Treatment of antiretroviral-naïve patients (Abstracts 138, 503, 506, 507)	
2 trials examining once-daily regimens, 1 with a once-daily lopinavir/ritonavir-based regimen and 1 with once-daily nevirapine regimen had suboptimal virologic response profiles.	No new once-daily options were evident from the data presented, although once-daily lopinavir/ritonavir regimens were comparable to twice-daily regimens in individuals with plasma HIV-1 RNA <5.0 log ₁₀ copies/mL.
Antiretroviral treatment strategies (Abstracts 513, 514, 516, 638)	
Lopinavir/ritonavir de-escalation was associated with continued suppression of plasma HIV-1 RNA for >1 year, but nonadherence consistently predicted loss of virologic suppression. Lamivudine monotherapy allowed patients with M184V mutations to remain off combination antiretroviral therapy for a longer period of time and was associated with fewer adverse events than treatment interruption.	Treatment interruption is associated with serious adverse events and de-escalation to monotherapy with lopinavir/ritonavir or lamivudine may be alternative short-term options for patients on long-term antiretroviral therapy who request treatment interruption.
Antiretroviral Resistance	
Summary	Potential Clinical Implications
Transmitted drug resistance (TDR; Abstracts 60, 648, 650, 653, 657)	
TDR occurs at relatively high frequencies in industrialized countries and if undetected can lead to initiation of inactive antiretroviral medications.	Baseline resistance testing in treatment-naïve individuals should precede and guide initiation of antiretroviral therapy. The natural history of TDR and its impact on clinical outcomes deserve further evaluation.
Low-frequency resistance variants (Abstracts 61, 639, 658, 666)	
Low-frequency resistance variants are not detected by standard resistance testing but appear to affect antiretroviral therapy response.	Additional studies should be conducted to determine the impact of low-frequency variants on outcome, and clinical cut-offs for sensitive resistance tests should be established. Future standard-of-care may include detection of low-frequency resistance variants to guide antiretroviral therapy but this is too labor intensive and too costly to be incorporated into practice in the near future.
Effect of subtype on resistance (Abstracts 59, 585, 624, 661, 664)	
Genetic variability of subtypes affects development of resistance mutations.	Clinicians should know the HIV-1 subtype of their patients, be familiar with resistance mutations associated with this subtype, and take these factors into consideration when choosing antiretroviral regimens. Additional research should be conducted to evaluate prevalence of resistance mutations among non-B subtypes, and their clinical implications, and databases and resistance algorithms should be updated accordingly.

Table 4: Key Findings and Potential Clinical Implications (continued)

Novel mutations in HIV-1 reverse transcriptase (Abstracts 90, 593, 594)

Mutations in the connection and RNase H domains of HIV-1 reverse transcriptase have only recently been evaluated and may represent novel resistance mutations.

Further studies should evaluate the impact of these mutations on resistance and fitness.

If these mutations prove to have clinical significance in addition to mutations already identified in the polymerase domain, routine sequencing of the entire HIV-1 reverse transcriptase may become standard in resistance testing.

Antiretroviral Treatment In Resource-limited Settings**Summary****Potential Clinical Implications****Treatment outcomes in large adult cohorts (Abstracts 33, 34, 35, 36LB, 62, 531, 535, 537)**

In resource-limited settings antiretroviral therapy initiation occurs at an advanced disease stage, active case-finding of patients lost to follow up is essential, and mortality within the first 3 months is high.

Addressing early mortality and loss to follow up remain important challenges in resource-limited settings.

Further options for second-line therapy are urgently needed.

Appropriate responses to first- and second-line antiretroviral treatment are observed, and adjusted mortality is comparable to that in some European and North American cohorts, but options for second-line therapy are very limited.

Treatment outcomes in large pediatric cohorts (Abstracts 79, 727, 728, 729, 732)

Children in resource-limited settings initiate treatment at a more advanced disease stage and at an older age.

Increased access to and earlier initiation of treatment are needed for children in resource-limited settings.

Response to therapy is encouraging, but anemia and low weight-for-age are predictors of mortality.

As recommended by the most recent World Health Organization guidelines, anemia and malnutrition should be considered in the decision to initiate antiretroviral treatment.

Laboratory monitoring in resource-limited settings (Abstracts 538, 531, 629, 673, 674)

Neither CD4+ cell count change over time nor a derived failure score performed well as substitutes for plasma HIV-1 RNA monitoring.

No clear alternative to monitoring plasma HIV-1 RNA in resource-limited settings exists.

Several new techniques for monitoring response to antiretroviral therapy in resource-limited settings are promising, but filter paper transfer of whole blood may lead to high false-positive rates.

Further development of less expensive, simpler assays is needed.

Adherence in resource-limited settings (Abstracts 530, 536, 548)

Nonadherence to antiretroviral treatment is high in some resource-limited settings.

Formal screening for adherence and addressing costs such as transportation and additional health care expenditures may improve adherence in resource-limited settings.

In addition to factors traditionally associated with decreased adherence in non-resource-limited settings, discontinuation of antiretroviral therapy in resource-limited settings may be related to transportation, other health care costs, and depression.

ter 2 of 2 subjects receiving efavirenz and the antimalarial drugs developed asymptomatic elevations in transaminases. The AUC of amodiquine was increased by 114% and by 302% in the 2 subjects and was the likely cause of the hepatitis.

Antiretroviral Exposure in Pregnancy. Peytavin and colleagues (Abstract 579) conducted a case-controlled study to evaluate the effect of pregnancy on lopinavir/ritonavir pharmacokinetics. Lopinavir trough levels were monitored in 100 HIV-infected women in the sec-

ond and in the third trimester and in nonpregnant controls. Trough levels were statistically significantly lower in pregnant women than in controls (C_{min} , 3806 and 3274 ng/mL in the second and third trimester, respectively, 5122 ng/mL in controls). Lower levels have

been associated with inadequate virologic suppression. In contrast, Khoung-Jones and colleagues (Abstract 743) found that lopinavir levels were adequate (C_{\min} , 5300 ng/mL) in 36 pregnant women who received the tablet formulation of lopinavir/ritonavir.

Burger and colleagues (Abstract 741) conducted an uncontrolled study of 14 pregnant women receiving saquinavir 1000 mg/ritonavir 100 mg twice daily plus 2 nRTIs. They found that all 14 women achieved adequate levels of saquinavir that were comparable with published data.

Ripamonti and colleagues (Abstract 742) presented data on 9 pregnant women receiving atazanavir/ritonavir during the third trimester of pregnancy. The pharmacokinetic parameters obtained during the third trimester were similar to those seen at 8 to 16 weeks postpartum. Cord blood levels of atazanavir were 220 ng/mL, approximately 10% that of concurrent maternal plasma levels. Natha and colleagues (Abstract 750) collected trough concentrations of atazanavir from 15 women receiving atazanavir/ritonavir during pregnancy and found a mean trough level of 421 ng/mL. All but 1 woman were above the minimum target level of 100 ng/mL.

Read and colleagues (Abstract 740) investigated the pharmacokinetics of nelfinavir among women in the third trimester of pregnancy compared with postpartum levels. Trough concentrations were lower during pregnancy and were suboptimal for most women at both time points. In addition, the metabolism of nelfinavir was statistically significantly altered during pregnancy. The AUC of M8, the virologically active metabolite of nelfinavir, was 80% lower during pregnancy than postpartum, further compromising the efficacy of this drug.

Antiretroviral Concentrations in Breastfeeding Infants. Substantial evidence was presented at this year's conference that formula feeding as a strategy for PMTCT of HIV is associated with a higher rate of mortality than is breastfeeding (see "HIV Epidemiology and Prevention Interventions" in this issue). The Kisumu breastfeeding

study in Kenya (Abstract 72) is a phase II, open label study providing nevirapine, lamivudine, and zidovudine in HIV-1-seropositive women for PMTCT during breastfeeding. An analysis of concentrations of antiretrovirals in maternal plasma, breast milk, and infant plasma in the 67 mother-infant pairs enrolled showed statistically significant variability of concentrations in each compartment. Maternal plasma and breast-milk concentrations of zidovudine were low (medians of 23 and 9 ng/mL, respectively) and median plasma concentration in infants was below the assay level of detection. Lamivudine concentrations were higher in breast milk and maternal plasma and the median plasma concentration in infants was 25 ng/mL, equal to the median inhibitory concentration (IC_{50}) but less than the optimal dose for virologic suppression. Nevirapine concentrations in maternal breast milk and plasma were even higher than that of lamivudine, and median infant plasma concentrations (911 ng/mL) were well above the IC_{50} but below the target dose for virologic suppression. The authors concluded that providing antiretroviral prophylaxis for mothers of breastfeeding infants may be effective for PMTCT but there are risks of adverse effects and the possibility of resistance in infants who become infected. The variable pharmacokinetics of antiretroviral use in nursing mothers warrants further research.

Conclusion

This year's conference maintained its reputation as the premier forum for presentation of new information in the field of antiretroviral therapeutics (see table 4). The likely additions of maraviroc and raltegravir, representing 2 new drug classes, to the list of FDA-approved antiretroviral drugs will improve our ability to maintain maximum virus suppression even in highly treatment-experienced patients. In addition, further clinical research involving these agents and others in their classes may well change current paradigms of therapy. Although the complexity of antiretroviral therapy and HIV-1 drug resistance is increasing,

data presented at this year's conference clearly demonstrate that public health approaches to delivery of antiretroviral therapy in resource-limited settings can be highly successful. Challenges in the field remain formidable but the basic and clinical research horizons in antiretroviral therapeutics are bright, and provide hope that these challenges can be met for the benefit of the nearly 40 million HIV-1-infected persons worldwide.

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