

Advances in Antiretroviral Therapy

Magdalena E. Sobieszczyk, MD, Angela K. Talley, MD, Timothy Wilkin, MD, MPH, Scott M. Hammer, MD

Antiretroviral therapy was a dominant theme of the 12th Conference on Retroviruses and Opportunistic Infections. Key focus areas were new drug advances, management strategies for treatment-naïve and treatment-experienced patients, the growing experience with antiretrovirals in the developing world, prevention of mother-to-child transmission of HIV, and the implications of HIV resistance. This review will highlight the major findings relevant to clinicians and clinical investigators.

Investigational and New Antiretroviral Agents

A summary of select investigational drugs is presented in Table 1.

Entry Inhibitors

CCR5 Antagonists. Demarest and colleagues presented data on GSK 873140, a CCR5 antagonist in phase 2 studies (Abstract 77). The short-term virologic efficacy data were presented previously (Demarest et al, ICAAC, 2004). The drug resulted in a 1.7- \log_{10} decline after being administered for 10 days, and the antiviral effect persisted 2 days after the compound was stopped. The authors examined samples from 31 participants in that study who received 1 of 4 doses tested (200 mg qd, 200 mg bid, 400 mg qd, and 600 mg bid) as well as 8 HIV-uninfected participants who received the compound (600 mg bid) for 7 days. The authors assessed the occupancy of the CCR5 receptors by GSK 873140 using a competitive monoclonal antibody to CCR5. They found that more than 98% of CCR5 receptors were occupied immediately after the last dose. The half-life for receptor occupancy was 122 hours across the different doses tested and was longest at the highest doses. It

did not differ between HIV-infected and HIV-uninfected participants. The prolonged receptor occupancy provides a reasonable explanation for the antiviral effect seen after the drug had been stopped and plasma levels of GSK 873140 were undetectable.

TAK-779 was a potent CCR5 antagonist in vitro whose clinical development was abandoned due to poor bioavailability of the drug. TAK-652 is an orally bioavailable derivative of TAK-779. Baba and colleagues presented data on the single-dose pharmacokinetics in HIV-uninfected volunteers (Abstract 541). The compound was well tolerated and achieved good plasma levels. They also found that TAK-652 had in vitro activity (50% inhibitory concentration [IC_{50}] < 1 nM) against a panel of CCR5-utilizing HIV-1 viruses that were resistant to either protease inhibitors (PIs) or reverse transcriptase inhibitors (RTIs) and viruses that were subtypes A through G. TAK-652 did not inhibit CXCR4-utilizing viruses. Tremblay and colleagues assessed the interaction of TAK-652 and other antiretrovirals in vitro (Abstract 542). They found that TAK-652 was additive with nucleoside RTIs (nRTIs), nonnucleoside RTIs (NNRTIs), and PIs. Interestingly, it was synergistic with enfuvirtide, suggesting a potential therapeutic benefit by targeting multiple steps of HIV entry.

Attachment Inhibitors. Attachment inhibitors are hypothesized to work by preventing the binding of gp120 to the CD4 receptor. Lin and colleagues presented a series of experiments that supported this mechanism of action (Abstract 544). They showed that a series of compounds, including BMS-488043, bound gp120 and prevented both the binding of soluble CD4 (sCD4) and the exposure of gp41. They also demonstrated confor-

mational changes in gp120 after binding BMS-488043. Finally, gp120 variants with mutations in the CD4 binding pocket were severely defective in compound binding.

Nonnucleoside Reverse Transcriptase Inhibitors

TMC278. Goebel and colleagues presented data on TMC278, a novel investigational NNRTI that is active against HIV-1 isolates resistant to currently available NNRTIs (Abstract 160). This was a double-blind, randomized, placebo-controlled trial of TMC278 given as monotherapy for 7 days. They enrolled 47 participants who were antiretroviral naïve, had a median CD4+ count of 255 cells/ μ L and plasma HIV-1 RNA level of 4.5 \log_{10} copies/mL. Participants started a standard combination antiretroviral therapy after completing 7 days of TMC278. The compound was well tolerated and no serious safety concerns were identified. The median change in plasma HIV-1 RNA at day 8 was 1.2 \log_{10} copies/mL across all dose groups, and no dose relationship was observed. The CD4+ count increased by an average of 55 cells/ μ L in the TMC278 groups, and no evidence of genotypic resistance was seen at day 8. This study supports further development of TMC278 as an addition to the NNRTI class of antiretroviral medications.

BILR 355 BS. Bonneau and colleagues presented data on BILR 355 BS, an NNRTI with potent in vitro activity (Abstract 558). They tested this compound against wild-type viruses and recombinant viruses with 1 or more NNRTI-associated resistance mutations. BILR 355 BS had a 50% effective concentration (EC_{50}) of 0.25 ng/mL against wild-type virus. The EC_{50} ranged from 1.5 ng/mL to 13 ng/mL against the NNRTI-resistant recombinant viruses. It was also active against subtypes A through G, but was inactive against HIV-2 similar to other NNRTIs. The investigators also presented data on the pharmacokinetic profile in HIV-uninfected volunteers given either a single

Dr Sobieszczyk is a Post-Doctoral Fellow at Columbia University College of Physicians and Surgeons, and Tisch Foundation HIV Fellow. Dr Talley is an Instructor in Clinical Medicine, Division of Infectious Diseases, at Columbia University Medical Center. Dr Wilkin is an Assistant Professor of Medicine, Division of International Medicine and Infectious Diseases, at Weill Medical College of Cornell University. Dr Hammer is the Harold C. Neu Professor of Medicine and Chief of the Division of Infectious Diseases at Columbia University Medical Center.

Table 1. Summary of Selected Investigational Drug Studies

Drug	Abstract Nos.	Drug Class	Development Stage	Results
BMS-488043	Abstract 544	Attachment inhibitor	Preclinical	Binding of drug to gp120 alters conformation of the envelope and inhibits CD4 attachment.
873140	Abstract 77	CCR5 antagonist	Phase 2	Drug occupies receptors with a half-life of 122 hours, which correlates with antiviral activity as opposed to plasma levels.
TAK-652	Abstracts 541, 542	CCR5 antagonist	Preclinical/Phase 1	EC ₅₀ <1 nM against a panel of clinical isolates; active against recombinant viruses of subtypes A-G; orally bioavailable.
KMMP05	Abstract 157	RNAase H inhibitor	Preclinical	IC ₅₀ 500 nM; binds near NNRTI binding site, not at RNAse active site.
Compound-1	Abstract 156	Nucleotide-competing reverse transcriptase inhibitor	Preclinical	EC ₅₀ 30 nM; binds at active site of reverse transcriptase but is not incorporated into DNA.
Amdoxovir	Abstract 553	nRTI	No longer in development*	Safe and well tolerated.
Dioxolane thymine	Abstract 554	nRTI	Preclinical	Increased activity against nRTI-resistant HIV, compared with wild type.
TMC278	Abstracts 160, 556	NNRTI	Phase 2a	Median decline in plasma HIV-1 RNA level was 1.2 log ₁₀ copies/mL after 7 days of monotherapy.
Capravirine	Abstract 555	NNRTI	Phase 2	See Table 3.
BILR 355 BS	Abstract 558	NNRTI	Preclinical	EC ₅₀ 0.26 ng/mL against wild-type HIV EC ₅₀ .
L-000870810	Abstract 161	Integrase inhibitor	No longer in development	1.7-log ₁₀ copies/mL reduction in plasma HIV-1 RNA level after 7 days of monotherapy.
Styrylquinolines derivatives	Abstract 547	Integrase inhibitor	Preclinical	Synergistic with reverse transcriptase inhibitors and diketo acid integrase inhibitors.
TMC114	Abstract 164LB	Protease inhibitor	Phase 2b	See Table 3.
Tipranavir	Abstracts 104, 560, 654	Protease inhibitor	Phase 3	See Table 3.
UIC-02031	Abstract 562	Protease inhibitor	Preclinical	Active against multiple PI-resistant strains of HIV with IC ₅₀ of 15-38 nM.
640385	Abstract 563	Protease inhibitor	Phase 1	Safe and well tolerated in non-HIV-infected volunteers. When boosted with ritonavir, achieves target plasma levels.
PA-457	Abstracts 159, 551	Maturation inhibitor	Phase 1	8 of 12 participants at 2 highest doses had 0.5-log ₁₀ copies/mL drop in HIV-1 RNA level after a single dose.

EC₅₀ indicates 50% effective concentration; gp120, glycoprotein 120; IC₅₀, 50% inhibitory concentration; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RNAase H, ribonuclease H.

*Although amdoxovir is no longer in development, the license has been returned to the original developer who may pursue further studies.

dose or multiple doses. The plasma half-life and maximum concentration (C_{max}) were increased by 3-fold to 5-fold with the coadministration of 100 mg of ritonavir. There was one transient grade 3 elevation of alanine aminotransferase (ALT), and no other safety concerns were noted. These data support further development of this compound.

Nucleoside Reverse Transcriptase Inhibitors

Amdoxovir. Gripshover and colleagues presented data on A5118: a randomized, placebo-controlled study of amdoxovir given with enfuvirtide plus an optimized background regimen (Abstract 553). The study was stopped early after a Data and Safety Monitoring Board review and the decision by the manufacturer not to develop the drug further. At that point, 9 patients were enrolled in each arm. The median baseline CD4+ count and plasma HIV-1 RNA level were 36 cells/ μ L and 4.8 log₁₀ copies/mL. Several patients stopped the study early because of poor virologic response and enfuvirtide injection-site reactions. The time-averaged change in HIV-1 RNA level was -1.1 log₁₀ copies/mL in the amdoxovir arm and -0.8 log₁₀ copies/mL in the placebo arm (*P* = not significant). The changes in creatinine clearance were similar between arms and no patients developed lens opacities, both potential toxicities of amdoxovir.

Dioxalane Thymine. Chu and colleagues presented data on dioxalane thymine, an nRTI with a dioxalane sugar moiety (Abstract 554). They found that this compound had an EC₅₀ of 0.43 μ M against wild-type HIV. The EC₅₀ was lower against nRTI-resistant strains, including those with K65R (0.21 μ M), L74V (0.33 μ M), and M184V (0.2 μ M) mutations. The authors concluded that these data support further clinical development.

Other Reverse Transcriptase Inhibitors

Nucleotide Competing Reverse Transcriptase Inhibitors (Compound-1) Jochmans and colleagues (Abstract 156) presented data on a potential new class of reverse transcriptase inhibitors: nucleotide-competing reverse transcriptase inhibitors. Compound-1 from this class was shown

to be a competitive inhibitor of reverse transcriptase with an EC₅₀ of 30 nM against HIV-1. Its structure is not similar to that of nucleotide reverse transcriptase inhibitors or nRTIs and is not incorporated into the DNA strand. It seems to bind to the active site of reverse transcription, thereby preventing the binding and incorporation of nucleotides into the growing DNA strand.

RNAase H Inhibitors. HIV requires a double-stranded DNA intermediate in order to replicate. Reverse transcriptase forms a complementary (-) DNA strand from a (+) RNA template. It also must form a (+) DNA strand from a (-) DNA strand to create the double-stranded DNA intermediate. RNAase H is the portion of reverse transcriptase that facilitates this transition from reading the (+) RNA strand to reading the (-) DNA strand, and is essential for HIV replication. Its activities include degrading the viral RNA strand. Progress in finding drugs that target RNAase H has been limited by the lack of a high-throughput assay that can easily screen thousands of compounds to find potential candidates. Parniak reviewed RNAase H and the process of finding RNAase H inhibitors in a conference symposium, which can be viewed on the Web site (<http://www.retroconference.org/2005/Pages/webcasts.htm>).

Himmel and colleagues presented data on the crystal structure of a candidate RNAase H inhibitor bound to the enzyme (Abstract 157). The compound, KMMP05, exhibited in vitro activity against RNAase H, but not reverse transcriptase, at an IC₅₀ of 500 nM. This compound bound at a site adjacent to the active site of RNAase H. Based on this, they speculated that this compound exerts its effect by diverting the RNA and preventing it from reaching the active site or by preventing other processes of the enzyme without directly binding in the active site. These data provide support for pursuing RNAase H inhibitors as a therapeutic class.

Integrase Inhibitors

L-000870810. HIV integrase is an important target for antiretroviral drug development. Little and colleagues presented data on a candidate integrase inhibitor, L-000870810, that inhibits HIV-1 integrase strand transfer in vitro (Abstract 161).

This was a double-blind, randomized, placebo-controlled trial of this compound given for 7 days. The study was stopped early because of toxicity seen in ongoing animal studies. Thirty patients who were not on antiretroviral therapy were enrolled. The participants who received 200 mg bid (*n* = 7) and 400 mg bid (*n* = 17) had a mean baseline plasma HIV-1 RNA level of 4.7 log₁₀ copies/mL and 4.6 log₁₀ copies/mL, respectively. They observed reductions in plasma HIV-1 RNA levels in the 2 groups of 1.7 log₁₀ copies/mL and 1.8 log₁₀ copies/mL, respectively, at day 8. The doses were well tolerated and no serious adverse events were noted. Although this compound is no longer in development, this study provides the first proof of concept for the antiviral activity of HIV integrase inhibitors, and a backup compound is now under development.

Styrylquinoline Derivatives. Styrylquinoline derivatives are integrase inhibitors that act on the preintegration step of HIV, most likely by preventing the formation of the preintegration complex. Chéret and colleagues presented data on the interaction of styrylquinoline derivatives with RTIs and diketo acids that inhibit the strand-transfer step of HIV integration (Abstract 547). These combinations were synergistic in vitro in supporting the use of styrylquinoline derivatives in multidrug regimens and the potential to target numerous steps in the integration process.

Protease Inhibitors

UIC-02031. Koh and colleagues presented data on UIC-02031, a nonpeptidic PI (Abstract 562). UIC-02031 was active against primary clinical isolates of HIV subtypes A, B, C, and E; clinical isolates resistant to available PIs; and PI-resistant laboratory isolates selected by suboptimal exposure to other PIs. UIC-02031-resistant isolates generated in vitro had L33F, M46I, V82I, and I84V mutations among others in the protease gene, and several cleavage site mutations in Gag.

640385. 640385 is a PI with potent in vitro activity against several PI-resistant isolates. Ford and colleagues presented data on the safety and pharmacokinetics of 640385 given to HIV-uninfected volunteers in a double-blind, randomized,

placebo-controlled, dose-escalating study (Abstract 563). They found that the pharmacokinetic profile was greatly enhanced by coadministration with ritonavir and supported twice-daily dosing. This compound appeared safe and well tolerated. All adverse events were listed as mild or moderate, and further studies in PI-experienced HIV-infected subjects are anticipated.

Maturation Inhibitors

PA-457. PA-457 is the first maturation inhibitor for the treatment of HIV infection to reach the clinical development phase. It inhibits the conversion of the HIV capsid precursor (p25) into the final capsid protein (p24), and results in non-infectious virions. Martin and colleagues presented the results of the first trial in HIV-infected individuals: a double-blind, placebo-controlled study of a single oral dose of PA-457 (Abstract 159). Participants were on no antiretroviral medications and had CD4+ counts above 200 cells/ μ L and plasma HIV-1 RNA levels between 5000 copies/mL and 250,000 copies/mL. They compared 3 doses of PA-457 (75 mg, 150 mg, or 250 mg) with placebo, with 6 participants in each group. All doses were well tolerated and no major safety concerns were identified. Eight of 12 participants in the 2 highest doses had at least a 0.3- \log_{10} copies/mL reduction in plasma HIV-1 RNA, and five had at least a 0.5- \log_{10} copies/mL reduction. The largest reduction was 0.7 \log_{10} copies/mL. Martin and colleagues also presented data from a separate study of 8 HIV-uninfected volunteers who received PA-457 (25, 50, or 100 mg qd) or placebo for 10 days (Abstract 551). They found that the doses were well tolerated and the pharmacokinetic profile supported once-daily dosing. Further development of this compound is planned. Freed reviewed the mechanism of action of PA-457 in an excellent talk about targeting the assembly and release of HIV (Abstract 116; <http://www.retroconference.org/2005/Pages/webcasts.htm>).

Treatment of Antiretroviral-Naive Patients

Bartlett and colleagues (Abstract 586) presented results of a metaanalysis of triple-combination therapy in antiretro-

viral-naive individuals. Forty-nine clinical trials, conducted between 1994 and 2004 and including 13,147 subjects in 85 independent treatment arms, were analyzed. Triple-combination therapy trials of at least 24 weeks in duration that had 30 or more chronically HIV-infected, antiretroviral-naive subjects, were included. The trials were selected from database searches and conference presentations. Triple-drug combinations included 2 nRTIs plus a PI, a boosted PI, an NNRTI, or a third nRTI. The primary endpoints were increase in CD4+ cell counts and proportion of subjects with plasma HIV-1 RNA levels below 50 copies/mL at week 48, as evaluated by intention-to-treat analysis. Overall, 57% of patients achieved an HIV-1 RNA level below 50 copies/mL at week 48 and an increase in CD4+ count of 177 cells/ μ L, both increased from the 2001 meta-analysis results of 45% and 158 cells/ μ L, respectively. Multivariate analysis of factors associated with response to therapy showed that lower pill count was no longer linked to improved response, and that boosted PI- and NNRTI-containing regimens were associated with virologic responses superior to nRTI-only or unboosted-PI regimens. Further, CD4+ response rates favored the boosted PI-containing regimens, with a significantly greater increase in CD4+ count (+209 cells/ μ L) than with the NNRTI- (+174 cells/ μ L), the triple-nRTI- (+150 cells/ μ L), or the unboosted-PI-containing regimens (+178 cells/ μ L). The investigators concluded that virologic response rates have been improving over time as better treatment regimens became available. Regimen potency, but not pill count, was associated with virologic response. Boosted PI and NNRTI regimens were associated with virologic suppression at 48 weeks superior to that of PI and nRTI regimens, and boosted PI regimens were associated with the greatest increases in CD4+ cell count.

INITIO Trial

The long-awaited results of the INITIO trial were presented by Cooper and Yeni for the INITIO Study Group (Abstract 165LB). The study compared the efficacy of a 3-drug regimen containing an NNRTI followed by a PI or a PI followed by an NNRTI with a 4-drug therapy containing an NNRTI and a PI in antiretroviral-naive

patients. This open-label, multicenter study randomized 915 people to receive: stavudine/didanosine/efavirenz followed by zidovudine/lamivudine/abacavir/nelfinavir after virologic failure; stavudine/didanosine/nelfinavir followed by zidovudine/lamivudine/abacavir/efavirenz after virologic failure; or stavudine/didanosine/efavirenz/nelfinavir with no specified second regimen after virologic failure. Drug switches were allowed for viral load rebound and for adverse events.

The primary outcomes were the proportion of patients with HIV-1 RNA below 50 copies/mL and change in CD4+ cell count from baseline at 3 years. Secondary outcomes measured included change from baseline in HIV-1 RNA at 3 years, progression to AIDS events or death, and incidence of adverse events. Subjects had been followed for a mean of 3.7 years when the trial closed in June 2004. The overall median baseline CD4+ count and mean plasma HIV-1 RNA level were 220 cells/ μ L and 4.93 \log_{10} copies/mL, respectively. Results analyzed on an intention-to-treat basis at 3 years favored the efavirenz arm, with virologic response rates of 74% (efavirenz), 62% (nelfinavir), and 62% (efavirenz/nelfinavir 4-drug arm) of patients with HIV-1 RNA levels below 50 copies/mL ($P = .004$) at 3 years. Proportions of time on the initial regimen were 74%, 63%, and 51%, respectively. No significant differences were found between groups in CD4+ cell response (mean increase 315 cells/ μ L, 289 cells/ μ L, and 274 cells/ μ L, respectively), in progression to a new AIDS event or death, in number of patients with serious adverse events, or number of patients with at least 1 adverse event leading to discontinuation of 1 or more drugs. Overall, 61% of patients stopped their initial regimen, usually in the setting of adverse events rather than virologic failure. However, patients in the efavirenz arm spent a longer proportion of time on the initial regimen and were least likely to be exposed to 3 drug classes over the 3-year period. Within-class switches occurred in 39% of all patients; the most common switch was from didanosine/stavudine to zidovudine/lamivudine. Thus, the findings of the INITIO trial support previous evidence from the AIDS Clinical Trials Group (ACTG) 384 study that initiating antiretroviral therapy with a 3-drug/2-class regimen containing efavirenz is

superior to starting treatment with similar regimens containing nelfinavir. Likewise, there remains no clear evidence to support the use of 4-drug/3-class therapy for the initial treatment of HIV infection. The nRTI backbone didanosine/stavudine was poorly tolerated, supporting the more favorable combination of zidovudine/lamivudine for use in initial treatment regimens.

Rizzardini and colleagues (Abstract 601b) compared 3-drug and 4-drug regimens in treatment-naive patients with respect to CD4+ count response, plasma HIV-1 RNA level, peripheral blood mononuclear cell (PBMC) proliferation, and cytokine production. Seventy-six treatment-naive individuals received zidovudine and were randomized to 1 of 6 arms: didanosine/abacavir, lamivudine/abacavir, didanosine/efavirenz, lamivudine/efavirenz, didanosine/indinavir/ritonavir, or lamivudine/indinavir/ritonavir. At 6 months, all regimens resulted in increased CD4+ cell counts and suppression of plasma HIV-1 RNA levels. The abacavir-containing regimens were associated with the best CD4+ cell response after 6 months of therapy, and the combination of zidovudine/lamivudine/abacavir resulted in better suppression of HIV-1 RNA levels. Triple-nRTI regimens overall resulted in higher increases in CD4+ cell counts than boosted PI regimens, yet the latter resulted in a more robust immune response as measured by interferon (IFN)- γ and PBMC proliferation.

ABCDE Study

Podzamczar and colleagues (Abstract 587) compared the efficacy and safety of 2 different nRTIs combined with lamivudine/efavirenz in treatment-naive subjects. This prospective, multicenter, open-label trial enrolled 237 patients with plasma HIV-1 RNA levels above 1500 copies/mL, who were randomized to receive either abacavir or stavudine in combination with lamivudine/efavirenz. The primary endpoint was lipodystrophy and mitochondrial toxicity, and the secondary endpoints were virologic, immunologic, and clinical efficacy and tolerability. Virologic success was determined by reduction of HIV-1 RNA level to below 50 copies/mL. Subgroup analysis was carried out at weeks 48 and 96 to assess further parameters of

lipodystrophy and mitochondrial toxicity including venous lactate, dual-energy x-ray absorptiometry (DEXA) scan, blood lipoproteins, mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratio. Baseline characteristics were similar between both groups, with a median CD4+ count of 213 cells/ μ L and HIV-1 RNA level of 5.2 log₁₀ copies/mL. At 96 weeks, abacavir was superior to stavudine in virologic response in the intent-to-treat analysis (60.9% vs. 47.5%; $P = .05$, but not in the on-treatment analysis (87.5% vs. 85.3%; $P = .81$) and demonstrated less-subjective and clinically-measured lipodystrophy (4.8% vs. 39.2%; $P < .0001$). These results were reinforced by the subgroup analyses that demonstrated superiority of abacavir in DEXA scan evaluation and lipid profiles, specifically lower triglyceride levels, greater high-density lipoprotein (HDL) and apolipoprotein A1 levels, and a greater reduction in total cholesterol/ HDL ratio. There were no differences in lactate levels, total cholesterol levels, low-density lipoprotein (LDL) levels, LDL/HDL ratio, or mtDNA/nDNA ratio between the 2 groups. The mean CD4+ cell count increases were likewise similar in both groups. The authors concluded, in this first head-to-head comparison of abacavir and stavudine, that abacavir is better tolerated, with less associated lipodystrophy, and they note that the lower treatment discontinuation rate in the abacavir group may account for the associated superiority in virologic response in this cohort. These results confirm findings of previous studies demonstrating the relationship between stavudine and mitochondrial toxicity.

nRTI Regimens in Treatment-Naive Patients

Current guidelines caution against initiating therapy with triple- nRTI regimens because of high rates of early virologic failure reported in treatment-naive patients treated with regimens containing tenofovir with either lamivudine/didanosine or lamivudine/abacavir. Recent data, however, suggest that this recommendation deserves ongoing evaluation (DeJesus et al, ICAAC, 2004, and Moyle et al, ICAAC, 2004).

Two studies evaluating nRTI-only regimens containing tenofovir plus fixed-

dose combination zidovudine/lamivudine were presented at the conference. Rey and colleagues reported data from a pilot, prospective, single-arm cohort study conducted at the University of Strasbourg (Abstract 599). Forty-two treatment-naive patients with CD4+ cell counts below 350 cells/ μ L received a fixed-dose combination of zidovudine/lamivudine (300 mg/150 mg bid) plus tenofovir (300 mg qd). Plasma HIV-1 RNA levels and CD4+ cell counts were assessed at 1 and 2 months and then every 2 months for 48 weeks; evaluation for early virologic response was assessed at weeks 1 or 2 of treatment. The median baseline CD4+ count was 233 cells/ μ L, and the median plasma HIV-1 RNA level was 4.88 log₁₀ copies/mL; 40% of patients had CD4+ counts below 200 cells/ μ L and 45% had plasma HIV-1 RNA levels above 5 log₁₀ copies/mL. The median time of follow-up was 8 months. On-treatment analysis showed median plasma HIV-1 RNA decreases of 1.56 log₁₀ copies/mL and 2.28 log₁₀ copies/mL at weeks 2 and 4, respectively. At week 4, 86% of subjects achieved plasma HIV-1 RNA levels below 1000 copies/mL; the median time to HIV-1 RNA level below 50 copies/mL was 10 weeks. The median increase in CD4+ count at 48 weeks was 82 cells/ μ L. Ninety-three percent and 78% of subjects had HIV-1 RNA levels below 50 copies/mL at weeks 24, and 48, respectively. Five patients (12%) discontinued the study regimen due to side effects (abdominal pain and nausea in 3 and anemia in 2, probably due to zidovudine). Four virologic failures occurred due to poor adherence. Genotypic analysis demonstrated the K65R mutation in 1 patient, the M184V plus 2 or 3 thymidine analogue mutations (TAMs) in 2 patients, and 2 TAMs in 1 patient present at baseline. The authors concluded that the combination of zidovudine/lamivudine plus tenofovir in treatment-naive HIV-infected patients induces a rapid and sustained virologic response and is associated with good immunologic response and safety profiles. The tenofovir-associated K65R mutation was not detected alongside TAMs in patients with virologic failure, suggesting that salvage options are available with alternate classes in this triple-nRTI regimen and the potential of triple-nRTI regimens merits further evaluation.

DART Substudy. Mutuluuza and colleagues (Abstract 22) presented results from the DART (Development of Anti-Retroviral Therapy in Africa) study supporting the viability of nRTI-only regimens for initial therapy. The DART trial is a large, randomized, controlled clinical trial of 3300 patients at 3 sites in Uganda and Zimbabwe comparing intensive vs clinical monitoring and continuous vs intermittent therapy in treatment-naïve individuals with a CD4+ below 200 cells/ μ L. Seventy-six percent of patients received fixed-dose zidovudine/lamivudine with tenofovir. Investigators evaluated virologic response to this regimen in a subset of 300 patients with advanced disease enrolled from early 2003 through October 2004. The median baseline CD4+ count was 100 cells/ μ L and the mean baseline HIV-1 RNA level was 300,000 copies/mL. At week 24, according to the intent-to-treat analysis, the mean decrease in plasma HIV-1 RNA level was 3.7 \log_{10} copies/mL and the median increase in CD4+ count was 106 cells/ μ L. Eleven patients died before week 24, and 47 of 281 (17%) had HIV-1 RNA levels above 1000 copies/mL at 24 weeks. Overall, after 24 weeks of therapy, 71% and 53% of subjects achieved an HIV-1 RNA level below 400 copies/mL and below 50 copies/mL, respectively. On-treatment analysis demonstrated virologic suppression in 83% of patients in whom treatment was not interrupted. The investigators concluded that this regimen has similar efficacy to PI-based or NNRTI-based regimens used in resource-unlimited settings. The authors also suggested that this triple-nRTI regimen may be particularly efficacious and well tolerated in populations with high rates of tuberculosis coinfection (27% in the DART study).

Treatment for Antiretroviral-Experienced Patients

Results of select studies for antiretroviral-experienced patients are summarized in Table 2.

TMC114 vs Control PI

Haubrich and colleagues presented data on TMC114, an investigational PI that is active against a broad range of HIV isolates with resistance to currently available PIs (Abstract 164LB). These data

were from a planned interim analysis of 2 ongoing dose-finding trials. Patients were 3-drug class experienced, had 1 or more major PI mutations, and had plasma HIV-1 RNA levels above 1000 copies/mL at screening. Subjects received TMC114 with ritonavir at 1 of 4 doses (400 mg/100 mg qd, 800 mg/100 mg qd, 400 mg/100 mg bid or 600 mg/100 mg bid) or a control PI chosen based on treatment history and resistance testing results. Participants also received an optimized background of nRTIs with or without enfuvirtide. The primary endpoint was change in plasma HIV-1 RNA level at week 24. Four hundred ninety-seven patients were included in the analysis. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.6 \log_{10} copies/mL and 141 cells/ μ L. Participants had a median of 8 PI mutations and 3 primary PI mutations. Phenotypic resistance to all currently available PIs was seen in 66% of patients. Forty-seven percent of patients used enfuvirtide in their optimized background regimen.

All doses showed significant declines in HIV-1 RNA levels compared with placebo. A dose-response relationship was seen, and the greatest HIV-1 RNA level decline, 1.85 \log_{10} , was seen in the 600 mg/100 mg twice-daily group. In addition, 47% of participants in this group had a plasma HIV-1 RNA level below 50 copies/mL at week 24, compared with 10% of participants in the control PI group. Among those participants receiving the highest dose of TMC114/ritonavir, 67% of those who also used enfuvirtide for the first time had a plasma HIV-1 RNA level below 50 copies/mL at week 24, compared with 37% who did not use enfuvirtide. These virologic responses were impressive given the level of treatment experience of the study population, but the durability of the response will need to be demonstrated. Further clinical development will use twice-daily doses of 600 mg of TMC114 given with 100 mg of ritonavir.

Tipranavir/ritonavir vs Lopinavir/ritonavir: A Subgroup Analysis of RESIST Trials

The RESIST-1 and RESIST-2 trials showed that the investigational PI tipranavir, boosted with low-dose ritonavir, given with an optimized background antiretro-

viral regimen was superior to an antiretroviral regimen using a currently available PI chosen based on history and resistance testing results. Patients were 3-drug class experienced, including 2 or more PI-based regimens. Enfuvirtide was allowed in the optimized background regimen. Cooper and colleagues presented a subgroup analysis of the trials that included only those participants choosing lopinavir/ritonavir for their comparator antiretroviral regimen (Abstract 560). Half of these participants were randomized subsequently to receive tipranavir/ritonavir. The baseline median CD4+ cell count and plasma HIV-1 RNA level were 162 cells/ μ L and 4.8 \log_{10} copies/mL. There were 293 participants included in the tipranavir/ritonavir arm and 290 in the lopinavir/ritonavir arm.

The proportion of subjects with greater than 1 \log_{10} copies/mL declines in plasma HIV-1 RNA level from baseline at week 24 was higher in the tipranavir group (40% vs 21%, respectively; $P < .05$). This difference was most apparent among those participants who had received lopinavir/ritonavir previously (35% vs 11%; $P < .05$) and those who had virus that was resistant to lopinavir/ritonavir (36% vs 13%, respectively; $P < .05$). The probability of a treatment response increased with the number of antiretroviral drugs to which the patients' isolates were susceptible, as has been noted in several other studies.

Placebo-Controlled Trial of Capravirine

Capravirine is an investigational NNRTI that is active in vitro against HIV-1 isolates that are resistant to currently available NNRTIs. Pharmacokinetic studies suggest that nelfinavir raises the plasma levels of capravirine. Pesano and colleagues presented the 48-week data from a phase 2, randomized trial of twice-daily capravirine 700 mg, capravirine 1400 mg, or placebo given with nelfinavir and 2 investigator-selected nRTIs (Abstract 555). Subjects were NNRTI-experienced and PI-naïve, and 60 subjects were enrolled in each arm. Mean baseline plasma HIV-1 RNA levels were 4.5 \log_{10} copies/mL, 4.4 \log_{10} copies/mL, and 4.4 \log_{10} copies/mL in the placebo, capravirine 700 mg, and capravirine 1400 mg groups, respectively, and median baseline CD4+ cell counts were

Table 2. Selected Antiretroviral Studies in Treatment-Experienced Patients

Abstract Number	Comparison	Baseline CD4+ count (cells/ μ L)	Plasma HIV RNA (copies/mL)	Length of follow-up (weeks)	Results
Abstract 164	TMC114/r (n=397)* vs investigator-selected control PI given with an optimized background regimen (n=100)	141	4.6 log ₁₀	24	TMC114/r 600 mg/100 mg bid was the optimal dose: 47% had a plasma HIV-1 RNA <50 copies/mL vs 10% in the control PI group
Abstract 577	Fosamprenavir/r or lopinavir/r (n=28) vs fosamprenavir+lopinavir/r (n=28) given with tenofovir and 1-2 nRTIs	188	4.5 log ₁₀	24	75% vs 61% with >1-log ₁₀ decline (P=.17) 54% vs 46% with HIV RNA <50 copies/mL
Abstract 578	Dual boosted PIs without RTIs (Group 1: resistant to RTIs, n=41; group 2: intolerant of RTIs, n=41)	240 (Group 1) 294 (Group 2)	4.1 log ₁₀ (Group 1) <400 (Group 2)	24	Overall at week 24, 84% and 91% had HIV-1 RNA levels below 400 copies/mL by intent-to-treat and on treatment analyses, respectively
Abstract 555	Capravirine 700 mg, 1400 mg or placebo given with nelfinavir and 2 nRTIs in NNRTI-experienced and PI-naive subjects (n=60 in each arm)	248 249 208	4.4 log ₁₀ 4.4 log ₁₀ 4.5 log ₁₀	48	43%, 58%, and 46% had a plasma HIV RNA <400 copies/mL
Abstract 560	Subgroup analysis of RESIST-1 and -2: tipranavir/r vs lopinavir/r given with an optimized background regimen	163	4.8 log ₁₀	24	40% vs 21% with >1-log ₁₀ decline in plasma HIV RNA

*4 different doses of TMC114/ritonavir were studied (400 mg/100 mg qd, n=100; 800 mg/100 mg qd, n=100; 400 mg/100 mg bid, n=98; 600 mg/100 mg bid, n=99)

NNRTI indicates nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside (or nucleotide) reverse transcriptase inhibitor; PI, protease inhibitor; r, boosted ritonavir; RTI, reverse transcriptase inhibitor.

208/ μ L, 248/ μ L, and 249/ μ L, respectively. The time to virologic failure did not differ between study arms. The proportions of subjects with plasma HIV-1 RNA levels below 400 copies/mL at week 48 were 46%, 43%, and 58% (P=not significant). The most common side effects were diarrhea, nausea, and vomiting, and did not differ between treatment arms.

Lopinavir/ritonavir or Fosamprenavir/ritonavir vs. Fosamprenavir plus Lopinavir/ritonavir

A prior report from A5143 showed that combining fosamprenavir and lopinavir/ritonavir led to a significant reduction of both lopinavir and fosamprenavir levels, compared with giving these drugs separately. Collier and colleagues presented the virologic data from that trial (Abstract

577). This study tested whether lopinavir/ritonavir 400 mg/100 mg plus fosamprenavir 700 mg twice daily (double PI) leads to HIV-1 RNA response superior to lopinavir/ritonavir or fosamprenavir/ritonavir (single PI) in persons with virologic failure to PI-based therapy. This was an open-label study that selectively randomized patients based on prior PI experience so that all patients received at least 1 new PI. All patients received tenofovir and 1 or 2 additional nRTIs chosen based on resistance testing results and antiretroviral history.

The median entry CD4+ cell count and plasma HIV-1 RNA level were 188/ μ L and 4.5 log₁₀ copies/mL, respectively. The study was stopped early based on the pharmacokinetic data, when 56 subjects (28 in the double-PI arm and 28 in the single-PI arm) were enrolled out of a planned sample size of

216 subjects. Seventy-five percent of participants in the double-PI arms had drops in HIV-1 RNA levels greater than 1 log₁₀ copies/mL from baseline at week 24, compared with 61% of participants in the single-PI arms (P=.17) in the intent-to-treat analysis, and 100% and 64% in the on-treatment analysis (P=.02). HIV-1 RNA levels were below 50 copies/mL at week 24 in 54% and 46% of the double-PI and single-PI subjects in the intent-to-treat analysis (P=.37) and in 75% and 48% in the on-treatment analysis. Although the virologic responses were not significantly different in the intent-to-treat analysis, the trends in the on-treatment analysis suggest that the reduction in plasma levels was not associated with adverse virologic outcomes. The question of whether dual-boosted PIs are superior to single boosted PI regimens remains unanswered.

Saquinavir/Ritonavir vs Indinavir/Ritonavir

Harris and colleagues presented data from the Simplified Protease Inhibitor Trial (SPRINT; Abstract 574). They compared once-daily saquinavir/ritonavir with twice-daily indinavir/ritonavir, each with 2 RTIs in patients who were either PI-naïve or had no evidence of PI resistance. They randomized 164 subjects to receive either saquinavir/ritonavir 1600 mg/100 mg once daily or indinavir/ritonavir 800 mg/100 mg twice daily, with either 2 nRTIs or 1 nRTI plus 1 NNRTI as selected by the treating physician. The efficacy analysis included 147 patients. Seventy-nine percent were male, 80% were PI-naïve, 62% had a plasma HIV-1 RNA levels above 5 log₁₀ copies/mL, and the median CD4+ count was 130 cells/μL. Background regimens included an NNRTI in 3 patients in the indinavir/ritonavir arm and none in the saquinavir/ritonavir arm. The proportion of patients with a plasma HIV-1 RNA level below 50 copies/mL in an intent-to-treat analysis were 56% and 49% in the saquinavir/ritonavir and indinavir/ritonavir arms ($P = .44$) at week 24, respectively, and 50% and 45% ($P = .7$) at week 48. CD4+ cell count increases to week 48 were also similar between arms (saquinavir/ritonavir, 147/μL increase; indinavir/ritonavir, 131/μL increase; $P = .60$). Patients taking indinavir/ritonavir were more likely to discontinue their PI because of toxicity (29 of 77 patients or 38%) than patients taking saquinavir/ritonavir (10 of 70 patients or 14%).

Dual-Boosted Protease Inhibitors Without nRTIs

Duvivier and colleagues presented data on a novel approach to antiretroviral therapy for treatment experienced patients: dual boosted protease inhibitors without use of reverse transcriptase inhibitors (Abstract 578). Subjects were either resistant to nRTIs and NNRTIs (group 1, $n = 41$) or were intolerant to those drugs (group 2, $n = 41$). The median baseline plasma HIV RNA was 4.1 log₁₀ copies/mL and the median baseline CD4+ count was 240 cells/μL in group 1. Seventy-one percent of those in group 2 had an HIV-1 RNA level below 400 copies/mL and 29% were on a treatment interruption. The median baseline CD4+ count

was 294 cells/μL in group 2. Subjects in both groups had prior exposure to a median of 2 PIs. Ten subjects in group 1 and 7 subjects in group 2 were PI-naïve. The most frequent regimens used were saquinavir 800 mg/lopinavir 400 mg/ritonavir 100 mg bid (40%), indinavir 400 mg/lopinavir 400 mg/ritonavir 100 mg bid (30%), and indinavir 400 mg/amprenavir 600 mg/ritonavir 100 mg bid (17%). Overall at week 24, 84% (69 of 82 patients) and 91% (67 of 76 patients) had HIV-1 RNA levels below 400 copies/mL by intent-to-treat and on-treatment analyses, respectively. Seven patients discontinued the study regimen before week 24 (5 in group 1 and 2 in group 2) and 5 patients experienced virologic failure at week 24 (4 in group 1 and 1 in group 2). This study adds to a series of data suggesting that boosted PIs without nRTIs or NNRTIs may be an adequate alternative to standard antiretroviral regimens in some settings.

Acute HIV Infection

Fiscus and colleagues (Abstract 20) evaluated methods of detecting acute HIV infection (AHI) among 1440 individuals evaluated between February 2003 and January 2004 in a sexually transmitted disease (STD) clinic in Malawi. Following 2 rapid tests, negative or discordant results were tested for HIV p24 antigen and pooled 1:10:50 for HIV-1 RNA testing. Acute infection was defined as the presence of HIV-1 RNA positive results and HIV-1 antibody negative results. Thirty-eight and a half percent of patients had established HIV-1 infection and 1.4% (20 patients) had AHI. Of these, only 50% had clinical signs and symptoms of AHI, with a median baseline plasma HIV-1 RNA level of 599,994 copies/mL. Sensitivity of two rapid tests was only 35% compared to 100% for HIV-1 RNA. The authors concluded that parallel rapid antibody testing and p24 antigen assay can detect up to 75% of cases of AHI.

Hightow and colleagues (Abstract 565) assessed risk behavior and clinical symptoms of 29 individuals with AHI identified through North Carolina Screening and Training Active Transmission program (STAT) between January 2003 and December 2004. Twenty three (85%) of the subjects reported having an acute illness in the 3 months prior to

testing; 19 of them sought medical treatment. The most common symptoms were fever (62%), anorexia (60%), sore throat (58%), and skin rash (38%). AHI was considered at only 3 (15%) of these initial evaluations. Sixty-two percent and 44% of subjects reported unprotected sex and symptoms of illness, respectively, as a reason for HIV-1 testing. The authors also reported (Abstract 566) that 38% of men were in college at the time of diagnosis, and 56% met their sex partners over the Internet.

Markowitz and colleagues (Abstract 973B) reported a case of a recent HIV infection by a multidrug-resistant and dual-tropic virus in a man who had sex with men and used methamphetamines. HIV-1 infection was confirmed in December 2004. The patient reported symptoms of pharyngitis, fever, and malaise in the 6 weeks preceding the diagnosis. Time of exposure was estimated to be between 4 and 20 months. The course of his illness over 4 months was characterized by presentation with symptomatic AIDS, a CD4+ count of 39 cells/μL and a plasma HIV-1 RNA of 232,000 copies/mL. The virus was noted to be resistant to nRTIs, some NNRTIs, and PIs; sensitive to enfuvirtide and possess a replication capacity of 136% and the ability to induce syncytia in PBMCs. Plasma virus utilizing both CCR5 and CXCR4 as the coreceptor were present.

The potential variations in the natural history of early HIV infection were highlighted by 2 presentations at the conference. Gange and Munoz (Session 31B) presented data on behalf of the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS). MACS and WIHS are prospective cohort studies of HIV-infected and high-risk individuals initiated in 1984 and 1994 that have enrolled 6,973 men and 3,768 women, respectively. In the MACS cohort, the median time to AIDS was estimated to be 8.3 years, and cumulative incidence of AIDS was 1.1% and 2.1% within 12 and 24 months of seroconversion, respectively. The authors used the MACS data to fit a model to estimate that the number of individuals progressing to AIDS at month 6, 12, and 24 of seroconversion would be 7, 45, and 262 per 10,000, respectively. Using both cohorts, the authors further estimated that the expected number of indi-

viduals with CD4+ counts below 200 cells/ μ L within 6 months of seroconversion would be 10 per 10,000.

In an analogous presentation, on behalf of the Tri-Service HIV/AIDS Consortium, Dolan described the variations in natural history of HIV-1 infection in a cohort of 4,500 United States military personnel. Since 1985 there have been 2,700 seroconverters. Of these, 12% achieved the endpoint of CD4+ count below 200 cells/ μ L or AIDS within 12 months of seroconversion.

Acute HIV Infection: Response to Treatment

It has been proposed that coadministration of cyclosporin A with antiretroviral agents during AHI leads to decreased immune activation (Rizzarda et al, *J Clin Invest*, 2002). Khonkarly and colleagues (Abstract 567) conducted an open label, prospective trial of 77 adults with AHI who were randomized to starting therapy (n=43) or starting therapy and an 8-week course of cyclosporin A (n=34). The mean baseline HIV-1 RNA level in the cyclosporin A and no-cyclosporin A arms were 5.8 log₁₀ copies/mL and 5.64 log₁₀ copies/mL, respectively and the mean baseline CD4+ counts were 480 and 461 cells/ μ L, respectively. At week 36 the proportion of patients with plasma HIV-1 RNA below 50 copies/mL was higher in cyclosporin A group (96% vs. 70%, respectively, $P=.011$). Comparing the 2 study arms, there were no differences in HIV-1 RNA changes during 120 weeks of follow up. The CD4+/CD8+ count ratio normalized more rapidly in the cyclosporin A arm ($P=.001$).

Benson and colleagues (Abstract 570) presented data on the safety and efficacy of antiretroviral therapy initiated during AHI and early (<3 months of seroconversion) HIV-1 infection. Fifty-five subjects were divided into 2 groups: starting atazanavir 600 mg (once daily)/didanosine/stavudine (n=37, 12 with AHI); and receiving no therapy (n=18, 3 with AHI). The overall median baseline HIV-1 RNA level was 50,950 copies/mL. At week 48, 66% of the treated group achieved plasma HIV-1 RNA levels below 50 copies/mL, the difference was not statistically significant between those initiating therapy during early infection versus AHI. At week 48, the median CD4+ counts were 725

cells/ μ L and 496 cells/ μ L for treated and untreated individuals, respectively ($P=.018$). Twenty-nine of 37 treated individuals (78.4%) developed grade 3 or 4 elevations of total bilirubin. Five had to discontinue atazanavir.

Primary Infection: Treatment Interruptions

Bloch and colleagues (Abstract 569) evaluated virologic response following structured treatment interruptions (STI) in patients receiving hydroxyurea. Sixty-nine patients with AHI (42%) and early infection were randomized into 2 groups starting indinavir/ritonavir/didanosine/lamivudine with or without hydroxyurea, followed by up to 3 STIs. The overall median baseline HIV-1 RNA level was 5.73 log₁₀ copies/mL. Fifty-nine patients underwent up to 3 STIs; of these, 18 maintained plasma HIV-1 RNA below 5000 copies/mL for 6 months off therapy. There were no statistically significant differences between treatment arms in the proportions of patients maintaining plasma HIV-1 RNA level below 50 copies/mL during an STI. During antiretroviral therapy, the mean CD4+ count increase from baseline was greater in the no-hydroxyurea arm (195 cells/ μ L) than in the hydroxyurea arm (100 cells/ μ L, $P=.014$). The number of serious adverse events was greater in the hydroxyurea arm (26% vs. 9%, $P=.11$). The authors concluded that hydroxyurea did not improve virologic outcome.

Superinfection

Grant and colleagues (Abstract 287) attempted to determine the frequency of HIV-1 sequentially expressed dual infections among 104 recently infected individuals. Highly divergent viral sequences appeared in 4 cases over time (incidence of 2.1/100 person years of observation). The authors used heteroduplex mobility assays and inspected electropherograms to confirm that the subsequent virus was not present in the baseline specimen; these methods have sensitivity of 1.5 to 3.0% for detection of minor sequence variants. Source partner recruitment will be attempted to determine if dual infection arose from sequential exposure and superinfections.

Plantier and colleagues (Abstract 288) described 1 patient initially infected with

an HIV-1 group O variant who was superinfected with an HIV-1 group M strain within 3 months of seroconversion. Analysis of the *env* regions of the virus demonstrated that the patient was infected with a group M CRF02_AG recombinant strain phylogenetically related to the partner's viral *env* region.

Treatment Strategies

Results of select treatment-strategy studies in antiretroviral-experienced patients are summarized in Table 3.

Fischl and colleagues (Abstract 162) presented the results of ACTG A5116, a randomized, open-label study enrolling 236 individuals with plasma HIV-1 RNA levels of 200 copies/mL or lower, previous PI or NNRTI experience, and no resistance mutations. Subjects were randomized to 1 of 2 treatment arms: lopinavir/ritonavir/efavirenz or efavirenz with 2 nRTIs. The composite endpoint was virologic failure (confirmed HIV-1 RNA above 2000 copies/mL) or toxicity-related treatment discontinuation. In the nRTI arm, 78% of patients received zidovudine/lamivudine, and 19% received stavudine/lamivudine. The median baseline CD4+ count overall was 475 cells/ μ L. The median follow-up time was 110 weeks. Overall, 70% (165) of subjects achieved plasma HIV-1 RNA level below 50 copies/mL: 74% in the nRTI arm and 66% in the lopinavir/ritonavir arm. Compared with the nRTI arm, there was a trend toward a higher rate of virologic failure ($P=.088$) and toxicity-related endpoints (17% vs 5%; $P=.002$) in the lopinavir/ritonavir arm, by intent-to-treat analysis.

Drug Reduction Strategy

Molina and colleagues (Abstract 573) presented the 3-year follow up of the ALIZE-ANRS 099 trial. This was an open-label, multicenter, noninferiority study enrolling 355 subjects with plasma HIV-1 RNA levels below 400 copies/mL who were on a stable PI-based regimen. Subjects were randomized to continue the initial regimen (n=177) or to switch to emtricitabine/didanosine/efavirenz once daily (simplification therapy, n=178). In the simplification arm, 152 patients (85%) completed week 48 of the study; of these, 147 (85%) were followed up until year

Table 3. Treatment Strategies in Antiretroviral-Experienced Patients

Study Name (Abstract No.) Description	Regimen/ Study Arm (No. of Patients)	Baseline HIV-1 RNA (copies/mL)	Baseline CD4+ (cells/ μ L)	Plasma HIV-1 RNA Response (copies/mL)	CD4+ Change (cells/ μ L)	Comments
ESS30008 (Abstract 572) 48-week multicenter, open-label, randomized, comparative trial Virologic failure was defined as week 48 plasma HIV RNA 0.5 log ₁₀ copies/mL increase over 400 copies/mL. All patients were abacavir/lamivudine experienced.	Abacavir/lamivudine fixed dose combination qd (n=130)	<50 (median)	565 (median)	81% with <50 at week 48	Not available	Most common third agent was efavirenz (62%), fosamprenavir/ritonavir (17%), and nelfinavir (14%). Grade 2-4 AEs were similar in both arms, and no abacavir hypersensitivity reactions were reported. 39% in the qd arm vs 31% in bid arm achieved >95% adherence.
	Abacavir, lamivudine bid (n=130)	<50 (median)	549 (median)	82% with <50 at week 48	Not available	
SPRINT (Abstract 574) 48-week prospective, randomized, open-label comparative trial Primary endpoint was week 24 and week 48 plasma HIV-1 RNA <50 copies/mL. Background regimen included 2 reverse transcriptase inhibitors.	Saquinavir 1600 mg/ritonavir 100 mg qd (n=70)	5 log ₁₀ (median)	152 (median)	56% with <50 at week 24, and 50% at week 48	+147 (median)	The indinavir arm had a higher discontinuation rate (51% vs 34%), most commonly due to renal and gastrointestinal AEs. Both arms sustained similar changes in lipid and glucose levels.
	Indinavir 800 mg/ritonavir 100 mg bid	5 log ₁₀ (median)	122 (median)	49% with <50 at week 24, and 45% at week 48	+131 (median)	
CPCRA 064 (Abstract 579) Randomized, prospective, clinical endpoint trial evaluating the impact of STI on HIV disease progression in patients with virologic failure Median length of follow-up was 36 months.	4-month STI (n=140)	5 log ₁₀ (mean)	183.3 (mean)	-0.8 log ₁₀ at month 24 (mean)	-3.2 (mean)	Total number of deaths were similar in both arms (30 in STI vs 33 in control); however, there were more progression of disease events in the STI arm (adjusted hazard ratio=1.66, P=.04).
	No STI (n=134)	5 log ₁₀ (mean)	177.5 (mean)	-0.8 log ₁₀ at month 24 (mean)	+39.6* (mean)	
CTN 164 (Abstract 580) 60-week, multicenter, open-label randomized trial to evaluate the impact of STI vs immediate salvage (IS) therapy on virologic outcome in patients with virologic failure Study was terminated by SERC because of slow recruitment.	12-week STI (n=67)	3.9 log ₁₀ (median)	320 (median)	-1.7 log ₁₀ at week 60 (median)	+25 (median)	There was no difference in proportion of patients maintaining plasma HIV-1 RNA level <50 copies/mL for 3 months (69% in IS vs 55% in STI arm, P=.11).
	IS (n=67)	3.9 log ₁₀ (median)	360 (median)	-1.7 log ₁₀ at wk 60 (median)	+94.5+ (median)	

continued, next page

Table 3. Treatment Strategies in Antiretroviral-Experienced Patients (continued)

Study Name (Abstract No.) Description	Regimen/ Study Arm (No. of Patients)	Baseline HIV-1 RNA (copies/mL)	Baseline CD4+ (cells/ μ L)	Plasma HIV-1 RNA Response (copies/mL)	CD4+ Change (cells/ μ L)	Comments
Enfuvirtide Salvage (Abstract 581) 48-week pilot study to evaluate virologic outcome of STI prior to enfuvirtide-based salvage regimen Patients had plasma HIV-1 RNA level >500 copies/mL and resistance to >2 PIs, >2 nRTIs, >1 NNRTIs.	16-week STI followed by enfuvirtide/optimized background (n=15)	4.79 log ₁₀ (median)	47 (median)	36% with <75 at week 24 and 48	Not available	Patients with virologic failure (return to <0.5 log ₁₀ copies/mL below baseline by week 16) had enfuvirtide resistance mutations in HR-1 sequence within 2 to 4 weeks of treatment.
	Immediate enfuvirtide/optimized background (n=15)	4.62 log ₁₀ (median)	26 (median)	53% with <75 at week 24 and 48†	Not available	

AEs indicates adverse events; bid, twice daily; HR1, helical region 1; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; qd, once daily; SERC, Safety and Efficacy Review Committee; STI, structured treatment interruption.

**P*=.07.

†*P*=.04.

‡Difference in virologic outcomes between study arms was not statistically significant.

3. The proportion of subjects with plasma HIV-1 RNA levels below 400 copies/mL in the simplification arm was 23% by intent-to-treat and 6% by on-treatment analysis. From month 12 to month 36, 27 patients in the simplification arm discontinued antiretroviral therapy due to patient choice (5 patients), treatment failure (2 patients), and adverse effect (8 patients). After 36 months of simplification therapy, there was no increase in total cholesterol level or LDL cholesterol level; 42% of subjects had plasma HDL cholesterol levels above 60 mg/dL. The authors concluded that the once daily regimen of emtricitabine/efavirenz/didanosine was not inferior to the PI-based regimen.

Sosa and colleagues (Abstract 572) presented results of ESS30008, a 48-week, randomized, open label, multicenter study comparing fixed-dose abacavir/lamivudine to twice-daily abacavir and lamivudine. The study enrolled 260 individuals with plasma HIV-1 RNA levels below 400 copies/mL and CD4+ counts of 50 cells/ μ L or above who were receiving abacavir/lamivudine twice daily with a PI or NNRTI. Subjects were randomized to continue abacavir and lamivudine twice daily or start fixed-dose abacavir/lamivudine. Virologic failure was defined as plasma HIV-1 RNA 0.5 log₁₀ copies/mL increase over 400

copies/mL (plasma HIV-1 RNA \geq 1265 copies/mL). The overall median baseline plasma HIV-1 RNA was below 50 copies/mL; the median baseline CD4+ counts were 565 cells/ μ L and 549 cells/ μ L in the once-daily and twice-daily arms, respectively. Ninety-two percent of subjects in the once-daily arm and 90% in the twice-daily arm completed the study. The most common third drugs were efavirenz (62%), fosamprenavir/ritonavir (17%), and nelfinavir (14%). Comparing the 2 study arms, there were no statistically significant differences in median CD4+ counts during follow-up, nor were there such differences in the proportions of patients with virologic failure, with HIV-1 RNA level below 50 copies/mL at week 48 (81% in the once-daily arm vs 82% in the twice-daily arm; *P*=.76). There were also no such differences in grades 2 to 4 adverse events: 2 subjects in the twice-daily arm withdrew due to weight gain and lymphoma that were not treatment related. Two subjects in the once-daily and 4 in the twice-daily arm met criteria for virologic failure after documented antiretroviral nonadherence. A greater proportion of subjects in the once-daily arm (39%) than the twice-daily arms (31%) maintained at least 95% adherence to the antiretroviral regimen.

Structured Treatment Interruptions in Antiretroviral-Experienced Persons

Lawrence and colleagues (Abstract 579) presented the final results of the CPCRA 064 study, a randomized, prospective study enrolling 274 individuals with virologic failure that evaluated the impact of STI on HIV disease progression. Subjects on a stable antiretroviral regimen who had plasma HIV-1 RNA levels above 5000 copies/mL and multi-drug-resistant HIV documented by genotypic testing were randomized to a 4-month STI followed by a salvage regimen (n = 140) or immediate initiation of a salvage regimen (n = 134). The primary endpoint was progression of HIV disease or death. The overall mean baseline CD4+ count and plasma HIV-1 RNA level were 180 cells/ μ L and 5.0 log₁₀ copies/mL, respectively; 58% of subjects had developed a prior opportunistic infection. At study entry, 96.7% of subjects had 3-class drug exposure, a median of 11 prior antiretroviral drugs and a mean of 9.7 drug resistance mutations. The median follow-up time was 36 months. At month 4, the mean changes in plasma HIV-1 RNA were a 0.4 log₁₀ copies/mL increase in the STI arm and 0.8 log₁₀ copies/mL decrease in the no-STI arm (*P*<.0001). Differences in plasma HIV-1 RNA levels at 24 months were not statistically significant. The mean change in

CD4+ count at months 4 and 24 were greater in the STI arm than in the no-STI arm; the difference persisted after 24 months (39.6 cells/ μ L increase vs 3.2 cells/ μ L decrease, respectively; $P = .007$). Subjects in the STI arm experienced a reduction in the number of mutations assessed (from 10 to 5.1) and 27% of subjects in the STI arm lost all mutations as assessed by standard sequencing of plasma present at study entry. There were no differences between the treatment arms in the total number of deaths: 30 in the STI and 33 in the no-STI arm ($P = .91$). The STI arm experienced more progression of disease events than did the no-STI arm, with 44 events in the STI arm and 29 in the no-STI arm (hazard ratio [HR] = 1.66; $P = .04$). The most common progression of disease event was esophageal candidiasis (26.3% and 10.4%, respectively) and *pneumocystis* pneumonia (12.3% and 6.3%, respectively). The authors confirmed that there was no benefit conferred by STI as part of salvage therapy in patients with advanced disease and multidrug-resistant HIV.

Henry and colleagues (Abstract 582) presented the results of ACTG A5102. This was a randomized pilot study enrolling antiretroviral experienced individuals with CD4+ counts above 500 cells/ μ L and plasma HIV-1 RNA levels below 200 copies/mL. Subjects were randomized to 1 of 2 arms: continue antiretroviral therapy with 3 5-day cycles of interleukin-2 (IL-2) administered every 8 weeks ($n = 23$); or continue antiretroviral therapy for 18 weeks. At the end of treatment period, subjects with CD4+ counts above 500 cells/ μ L underwent a treatment interruption (TI) until the CD4+ count dropped below 350 cells/ μ L. The median follow-up on TI was 78 weeks; the median baseline CD4+ count overall was approximately 750 cells/ μ L. Subjects in the IL-2 arm maintained higher CD4+ counts for 72 weeks during the TI. A higher CD4+ count before antiretroviral therapy was associated with a longer time to CD4+ count below 350 cells/ μ L. Higher plasma HIV-1 RNA level in the beginning of TI was associated with shorter time to CD4+ count below 350 cells/ μ L (HR = 3.88; $P = .042$).

Deeks and colleagues (Abstract 680) evaluated the effect of stopping enfuvirtide therapy in patients with enfuvirtide resistance. There were 22 subjects in whom enfuvirtide was failing. The mean

baseline plasma HIV-1 RNA level was 5.08 log₁₀ copies/mL, and mean duration of enfuvirtide therapy was 28.6 weeks; these subjects stopped enfuvirtide while continuing background antiretroviral therapy. At weeks 2 and 4, the mean increases in plasma HIV-1 RNA level were 0.11 log₁₀ copies/mL ($P = .3$) and 0.27 log₁₀ copies/mL ($P = .03$), respectively. At baseline, clonal analysis of HR-1 sequences documented the presence of enfuvirtide-associated mutations in 21 of 22 patients. At week 16, enfuvirtide mutations were no longer detectable in clones from the majority of subjects ($P = .003$). The authors concluded that this is consistent with a fitness cost associated with enfuvirtide resistance.

The Emergence of Resistance During STIs

Ceccherini-Silberstein and colleagues (Abstract 681) investigated the disappearance of PI mutations during TI in 88 patients with at least 2 genotypes within 1 year: 1 during PI-containing regimen failure and 1 during TI (median length, 4 months). Mutations L33F, I47V, F53L, G73S, N88D, M46I/L and L90M disappeared in 33%, 66%, and 99% of patients at 6-, 9-, and 12-month intervals during TI, respectively. Mutations K20T, K43T, Q58E, T74S, I85V, Q92K, and C95F disappeared at a similar rate during TI. Mutations M36I, L63P, V77I were maintained in more than 75% of patients during TI. At month 3 of TI ($n = 23$), major PI mutations reverted back to wild type in 14 out of 23 patients (61%); among these, there was a trend toward lower plasma HIV-1 RNA level ($P = .08$). The disappearance of M46I and L90M was associated with greater plasma HIV-1 RNA increase and greater CD4+ cell decrease ($P < .001$). Maintenance of M36I, L63P, and V77I compensatory mutations was associated with higher plasma HIV-1 RNA increase ($P < .001$). The authors concluded that M46I and L90M confer a disadvantage for viral fitness.

Antiretroviral Drug Resistance and Replicative Capacity

Transmission of Drug-Resistant Virus

On behalf of the Duke-University of North Carolina-Emory Acute HIV Consor-

tium and the STAT program, Hicks (Abstract 673) presented data on genotypic resistance profiles of virus from 127 individuals with AHI and early infection diagnosed between January 1998 and December 2004. Overall, major resistance mutations were detected in 19.7% of cases and they did not differ significantly among the 3 major classes of antiretroviral agents: 7.1% for nRTI, 6.3% for NNRTI, 3.1% for PI. Prevalence of resistance mutations was similar among AHI and early infections.

In a complementary presentation, De Mendoza and colleagues (Abstract 672) documented a reduction in the rate of transmitted drug resistance among 198 consecutive newly HIV-infected individuals seen between January 1997 and December 2004 in 11 hospitals in Spain. The overall rate of genotypic resistance in this population was 12.1%, and 9.3%, 4.1%, and 2.1% had nRTI, NNRTI, or PI resistance mutations, respectively. The rates of drug resistance in 1997, 2003, and 2004 were 33.3%, 10.4% and 8.2%, respectively. The authors also noted a rise in the transmission of non-B subtypes of HIV-1.

Shet and colleagues (Abstract 289) described the transmission of drug-resistant HIV among 112 individuals with AHI presenting to the Aaron Diamond AIDS Research Center in New York between 2003 and 2004. The median duration of infection was 58 days, 98% were men who have sex with men (MSM). Of them, 27 individuals (24.1%) had drug-resistant HIV-1. Compared with the period between 1995 and 1998, NNRTI resistance increased from 2.6% to 13.4% in 2003 through 2004 ($P = .04$), PI resistance increased from 1.3% to 7.1% in 2003 through 2004 ($P = .09$). The prevalence of nRTI-associated mutations has remained stable since 2000.

The role of genital tract shedding in transmission of drug resistance was highlighted by several presentations at the conference. Katzenstein and colleagues (Abstract 670) presented the results from A5077, a 96-week, multicenter, longitudinal study evaluating genotype of plasma virus associated with genital tract shedding. The study enrolled 88 individuals with plasma HIV-1 RNA levels more than 2000 copies/mL, with matched genital tract samples for whom genotypic susceptibility score (GSS) was obtained within 10 days ($n = 29$) or 90

days ($n=53$) of starting antiretroviral therapy. Overall, subjects had a median of 5.6 years of prior antiretroviral therapy and a median plasma HIV-1 RNA level of $4.7 \log_{10}$ copies/mL. Among these highly drug-experienced patients, more than 50% of women had resistance to more than 2 NNRTI drugs; men tended to have less NNRTI resistance and more PI resistance (GSS, 2.05 and 1.45, respectively; $P=.08$). Overall, the frequency of genital tract HIV shedding was 10-fold higher among men receiving antiretroviral therapy than women. The authors concluded that these observations may explain the increasing frequency of drug resistance among newly infected MSM.

Newstein and colleagues (Abstract 671) aimed to describe drug resistance in plasma and genital tracts of NNRTI-experienced women failing antiretroviral therapy. In 7 out of 8 women, NNRTI mutations were detected in both plasma and genital samples; K103N was detected in both sites in 4 out of 5 patients who had been off therapy for up to 28 months. The authors concluded that NNRTI mutations are stable in the female genital tract in the absence of drug selection.

Treatment-Experienced Patients

Didanosine. Bates and colleagues (Abstract 105) described the relationship between baseline phenotypic susceptibility and week-4 HIV-1 RNA level response to didanosine in the JAGUAR study. This was a multicenter, randomized, double-blind, placebo-controlled study of 168 individuals with plasma HIV-1 RNA levels between 3 and $5 \log_{10}$ copies/mL and CD4+ counts above 100 cells/ μ L, who were randomized to add didanosine ($n=111$) or placebo ($n=57$) to a current, stable regimen. At week 4, the median changes in HIV-1 RNA in the didanosine and control arms were a $0.56 \log_{10}$ copies/mL decrease and a $0.07 \log_{10}$ copies/mL increase, respectively ($P<0.0001$). The highest mean \log_{10} change from baseline in plasma HIV-1 RNA levels occurred in individuals with a didanosine fold change (FC) less than 1.3 ($-1.01 \log_{10}$ copies/mL), compared with those with a didanosine fold change greater than 2.2 ($-0.24 \log_{10}$ copies/mL; $P<0.001$). At a didanosine fold change less than 1.3, individuals experienced high response rates, while

at a fold change greater than 2.2, the response rates were considerably lower. The authors proposed clinical cutoffs at 1.3 and 2.2 for didanosine to identify a zone identifying the probability of an intermediate virologic response.

nRTI-Associated K65R Mutation. The K65R mutation reduces TAM-mediated excision of zidovudine and is responsible for reversal of zidovudine resistance. Parikh and colleagues (Abstract 98) attempted to demonstrate that K65R and 215Y/F do not exist on the same genome. A total of 59,262 samples from a commercial database with K65R in combinations with 2 or more TAMs were analyzed. Of these, 3.2% had K65R and 14.2% had 215Y/F or 2 or more TAMs. The expected frequency of having both K65R and TAMs was 0.5% and the actual frequency of both was 0.04%. Single genome sequencing was performed on 173 genomes from 10 samples; K65R and any TAM occurred together in less than 10% of the samples and among these K65R was never found on the same genome with T215FY/F/I.

Atazanavir. Coakley and colleagues (Abstract 716) described a PI-naïve patient who developed the N88S mutation without the sentinel atazanavir mutation I50L on atazanavir/ritonavir therapy. The patient had a baseline plasma HIV-1 RNA level of 6547 copies/mL, replicative capacity of 96%, and no baseline PI mutations when he started on atazanavir/tenofovir/abacavir/lamivudine. Ritonavir was added at month 3, plasma HIV-1 RNA level less than 50 copies/mL at month 4, and increased to 7535 copies/mL at month 11. The genotype at that time demonstrated K20T, M36I/V, L63P, A71T, and N88S mutations. The fold changes to atazanavir, amprenavir, indinavir, lopinavir, nelfinavir, and saquinavir were 56, 0.3, 13, 4.3, 68, and 4.2 respectively. The replicative capacity was 14%. In one commercial database, the N88S mutation was associated with resistance to atazanavir, nelfinavir, and indinavir, susceptibility to lopinavir, and ritonavir, and hypersusceptibility to amprenavir. Isolates with I50L mutations, on the other hand, demonstrated atazanavir-specific resistance, susceptibility, and hypersusceptibility to ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, and saquinavir.

Surveillance of the database revealed that among samples with at least 1 major PI mutation, the prevalence of N88S and I50L increased from 1.04% and 0.15% in December 2003, to 2.86% and 3.65% in January 2005, respectively.

Tipranavir/Ritonavir. Schapiro and colleagues (Abstract 104) evaluated the resistance profile of tipranavir/ritonavir in the RESIST-1 and RESIST-2 trials. Patients with 3-class experience and 1 or more major PI mutations (among 30N, 46I/L, 48V, 50V, 82A/F/L/T, 84V, and 90M), were randomized to tipranavir/ritonavir ($n=582$) or a comparator PI/ritonavir (CPI/r; $n=847$). At baseline, 59% of patients in the tipranavir arm and 61% in the CPI/r arm had 3 or 4 primary PI mutations. The primary endpoint was defined as a confirmed $1 \log_{10}$ copy/mL decrease in plasma HIV-1 RNA level from baseline. At week 24, patients in the tipranavir/ritonavir arm had better treatment responses than those in the CPI/r arm regardless of the number of baseline protease gene mutations. Among subjects with 12 or less and 19 or more protease gene mutations, 50.4% versus 29.8% and 31.7% versus 7.7% of subjects in the tipranavir/ritonavir and CPI/r arms, respectively, reached the primary endpoint of more than $1 \log_{10}$ copy/mL decrease in plasma HIV-1 RNA from baseline. Twenty individuals had what was described as PI-resistance-associated mutations (PRAMS): L33W/F, V82A/F/L/T, I84V and L90M. Among these there was no difference in virologic response between arms in subjects with 0 PRAMS; however, with 1 and 2 PRAMS, 44% versus 25% and 41% versus 15% in the tipranavir/r and CPI/r arms, respectively, achieved treatment response.

Enfuvirtide. Enfuvirtide resistance is associated with changes at amino acid positions 36 to 45 of the N-terminal region of the first heptad repeat (HR1) in the envelope gene. Labrosse and colleagues (Abstract 97) investigated the evolution of enfuvirtide resistance in 6 patients with different degrees of susceptibility on enfuvirtide-containing salvage therapy. Baseline susceptibilities to enfuvirtide ranged from 56 ng/mL to 756 ng/mL. Mutations emerged at positions 36, 38, and 43 in the context of *env* quasispecies different from dominant

baseline populations, and were not accompanied by loss of *env*-dependent activity. In 4 patients, HIV-1 fitness increased from baseline. The authors concluded that mutations conferring enfuvirtide resistance are selected repeatedly in different *env* genetic backgrounds, resulting in replacement of dominant virus populations over time.

Maroldo and colleagues (Abstract 717) described the rapid selection of enfuvirtide resistance due to a single V38E mutation within the enfuvirtide binding site, appearing on day 9 of therapy. Mutations G36G/D and V38V/E/A were noted in the HR1 domain; clonal analysis of the gp160 *env* demonstrated that the G36D mutation was present on distinct strains from those carrying V38A or V38E. Samples from day 580 of therapy demonstrated persistence of only the V38E mutation. High level phenotypic resistance and reduced viral fitness were previously described with the V38E mutation in the context of N42S (Lu et al, *J Virol*, 2004). The authors concluded that the rapid outgrowth and persistence of V38E is consistent with a high selective advantage of this mutation.

Cabrera and colleagues (Abstract 718) described sequential accumulation of mutations in the gp41 *env* region after long-term enfuvirtide therapy. Samples were derived from 15 highly treatment-experienced individuals with virologic failure on enfuvirtide-containing salvage regimens. Mutations in HR1 and HR2 were analyzed by population-based sequencing. Mutations G36D/S, V38A, and N43D appeared 2 to 4 weeks after initiation of enfuvirtide. Double mutations V38A/G36V, V38A/N43D, G36V/N43D were established early in the treatment but never coexisted in the same viral genome.

UK-427,857. UK-427,857 is an investigational CCR5 blocker that binds to CCR5 through a triazole group. Virus resistant to UK-427,857 has mutations in the V3 loop. Westby and colleagues (Abstract 96) identified 8 CCR5 antagonists structurally related to UK-427,857 and investigated their binding sites and potential for developing cross-resistance. These compounds bind CCR5 in a pocket formed by the transmembrane helices and extracellular loop 2. UK-427,857-resistant variants are cross-resistant to compounds with a triazole functional

group, but not with an imidazopiperidine group. The authors concluded that resistance to CCR5 antagonists may not lead to drug-class resistance.

Interactions Among RTI Resistance Mutations

Two presentations focused on interactions of L74V with other mutations. Frankel and colleagues (Abstract 698) used real-time polymerase chain reaction (PCR) to demonstrate that L74V was associated with a 50% reduction in the efficiency of synthesis of viral DNA and excision of zidovudine-terminated DNA synthesis. The presence of M184V further potentiates this effect. The authors concluded that M184V, K65R, and L74V share a common mechanism of resistance to nRTIs and their effects on reverse transcriptase.

Miranda and colleagues (Abstract 699) demonstrated that L74V interferes with the primer unblocking in the presence of TAMs, though to a lesser extent than M184V. The effect of M184V on primer unblocking was greatest in viruses with M41L, L210W, T215Y, and impaired in the presence of 6 TAMs. The authors concluded that this is the mechanism by which L74V partially reverses TAM-mediated zidovudine resistance.

Several posters presented data about the effects of interactions between nRTI and NNRTI mutations on HIV-1 replicative capacity and fitness. It was previously noted in ACTG 368 that K103N plus L100I were frequently associated with L74V in the nRTI-sparing arm of the study. L100I is selected for during efavirenz therapy and adds to the resistance of K103N. Koval and Demeter (Abstract 700) used growth competition experiments in H9 cells to elucidate whether L74V compensates for the replicative fitness defect seen in the K103N/L100I double mutant. The K103N/L100I double mutation was associated with reduced fitness compared with K103N ($P < .0001$), and the relative fitness of the L74V triple mutant (L74V/K103N/L100I) was significantly higher than the K103N/L100I double mutant.

Colson and colleagues (Abstract 701) investigated the frequency of the K65R/L74V double mutant in the Marseille database. This was a retrospective analysis of 3201 patients and 7151

sequences: 12 of 3201 patients carried the K65R/L74V combination of mutations and 4 of these had virologic failure on a nRTI-based regimen and no protease resistance mutations. This is contrary to in vitro data reporting diminished replicative capacity and dysfunctional reverse transcriptase in the presence of K65R/L74V.

Wirten and colleagues (Abstract 702) analyzed linkage of K65R mutation with TAMs and L74V in 5 samples from patients obtained after tenofovir failed. Clonal analysis revealed association of K65R with M41L, D67N, L210W, and K219E, but no linkage between K65R with L74V.

Coakley and colleagues (Abstract 704) evaluated the effect of non-thymidine analogue nucleoside analogue mutations (non-TA NAMs) on efavirenz hypersusceptibility, defined as fold change in IC_{50} of less than 0.4. Samples from a commercial database with unmixed nRTI mutations (K65R, T69X, I74I/V, V75X, M184I/V; X = any amino acid change) but no TAMs (ie, none of M41L, D67N, K70R, L210W, T215FY, K219X) and no Q151M, T69 insertions, or NNRTI mutations were identified. Isolates without nRTI, NNRTI, and PI mutations served as the control, wild-type group. The mean efavirenz fold change was lower in isolates containing non-TA NAMs or TAMs than in control isolates. Over 40% of isolates with K65R, K65R/M184V double mutants, or 3 TAMs (including T215Y) had fold change in efavirenz IC_{50} of less than 0.4. The authors concluded that efavirenz hypersusceptibility is associated with non-TA NAMs as well as TAMs.

Resistance in Non-Subtype B Infections

Grossman and colleagues (Abstract 719) compared virologic response and resistance to lopinavir/ritonavir between clade C and clade B HIV-1 viruses. Samples were obtained from 37 subtype-B and from 49 subtype-C individuals with mean PI-treatment duration overall of 20 months. The mean plasma HIV-1 RNA levels were 5.14 \log_{10} copies/mL and 5.24 \log_{10} copies/mL in subtype C and B groups, respectively. Mutations were present with the following prevalence in clade C and B patients, respectively: 49% and 57% with L101V/F; 55% and 30% with K20R ($P = .03$); 4% and 14%

with L24I ($P = .07$); 33% and 32% with M46I; 39% and 46% with I54V; 33% and 92% with L63P ($P < .001$); 22% and 41%, with A71V ($P < .001$); 43% and 35% with V82A; 10% and 24% with I84V; 27% and 14% with L90M; and 98% and 46% with L93I. Mean lopinavir/ritonavir mutational score was 3.29 in subtype-C and 4.05 in subtype B, and the difference was not statistically significant. The authors concluded that the prevalence of lopinavir-associated mutations is similar in clade B and C patients in whom therapy is failing. Differences in frequencies of mutations in codons 20, 24, and 63, may be due to baseline polymorphic variations between subtypes.

Bessong and colleagues (Abstract 721) analyzed resistance mutations in the protease region of 40 antiretroviral-naive, subtype C-infected individuals from South Africa. No major PI mutations were detected; M36I and I93L were detected in 90% of the sequences; the combination of K20R and M36I mutations conferring indinavir and ritonavir resistance were detected in 25% of isolates.

Fitness and Replicative Capacity

Hicks and colleagues (Abstract 345) described an inverse relationship between baseline replicative capacity and CD4+ count among 132 antiretroviral-naive individuals who achieved plasma HIV-1 RNA level below 400 copies/mL after 12 months of antiretroviral therapy. The mean baseline CD4+ count was 223 cells/ μ L and mean plasma HIV-1 RNA level 4.9 \log_{10} copies/mL. There were 52% and 37% of individuals who received a combination of NNRTI with a NRTI or NRTI with a PI, respectively. Baseline replicative capacity was inversely correlated with baseline CD4+ count ($P = .003$). After adjusting for baseline CD4+ and plasma HIV RNA level, for every increase of 1% in the replicative capacity, there was a corresponding decrease in baseline CD4+ count of 0.88 cells/ μ L ($P = .049$).

Martinez-Picado and colleagues (Abstract 691) examined the relationship between hypersusceptibility phenotype to multiple PIs and HIV-1 replicative capacity among 12 patients undergoing 5 consecutive STIs. Samples were collected at the peak of viremia during each STI. Ten patients had at least 1 sample demonstrating hypersusceptibility to 1

or more PIs. The median drug susceptibility overall for amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir was 0.2 fold-change lower than the median drug susceptibility for wild-type virus from the a commercial database. The mean replicative capacity of recombinant virus containing the 3'-end of *gag*, the protease and reverse transcriptase regions of all the isolates was 53% lower than expected for wild-type virus, and there was significant correlation between susceptibility to PIs and replicative capacity in all 12 patients. Replicative capacity of the virus did not change during the STIs, and there was no apparent association between replicative capacity as measured in vitro and CD4+ count, CD8+ count, and plasma HIV-1 RNA level at each treatment interruption.

De Luca and colleagues (Abstract 692) evaluated the relationship between treatment outcome and HIV-1 replicative capacity in 139 patients from the ARGENTA trial. Patients included those in whom antiretroviral therapy was failing; the mean plasma HIV-1 RNA level was 4.28 \log_{10} copies/mL and the median CD4+ count was 264 cells/ μ L. At baseline, the median replicative capacity was 59% and the median phenotypic susceptibility score (PSS) of the first salvage regimen was 2. At month 36, higher PSS predicted better virologic response; the mean decrease from baseline plasma HIV-1 RNA of 1.6 \log_{10} copies/mL and 0.8 \log_{10} copies/mL for PSS of 2 or greater and PSS less than 2, respectively ($P = .040$). Replicative capacity was inversely correlated with decreased susceptibility to PIs. Among 85 patients with persistent plasma HIV-1 RNA below 500 copies/mL, after adjusting for PSS, replicative capacity above 65% was associated with lower CD4+ count gains at month 3 (mean decrease of 58 cells/ μ L; $P = .04$); month 9 (mean decrease of 147 cells/ μ L; $P = .003$), and month 24 (mean decrease of 125 cells/ μ L; $P = .047$). Among individuals with PSS of 3 and on their first salvage regimen ($n = 25$), a 1-log higher replicative capacity was associated with higher plasma HIV-1 RNA levels at 3 months (mean plasma HIV-1 RNA increase from baseline of 1.52 \log_{10} copies/mL; $P = .019$). In a univariate analysis, a log change in replicative capacity was not associated with clinical progression (HR,

0.79; $P = 0.66$). The authors concluded that in persistently viremic patients, higher replicative capacity predicted worse 3-month virologic and 24-month CD4+ count outcomes.

Predictors of Response

On behalf of the British Columbia Center for Excellence in HIV/AIDS, Hogg (Abstract 712) described the effect of increasing antiretroviral resistance on survival among 1388 individuals initiating antiretroviral therapy between August 1996 and July 2000. During a median follow-up time of 52.7 months, 238 individuals died (17.2%, all-cause mortality). Resistance testing was available on 3120 samples; of these, drug resistance to at least 1 class and all 3 classes of antiretrovirals was noted in 28.5% and 7.9%, respectively. After controlling for age, CD4+ count, and plasma HIV-1 RNA level, NNRTI and NRTI resistance other than the presence of M184V, was associated with an increased risk of death (odds ratios [ORs], 2.07 and 2.93, respectively), and development of PI resistance was protective (OR, 0.32).

Previous studies have suggested that TAMs develop by 1 of 2 distinct pathways described as TAM1 (41L, 210W, and 215Y) and TAM2 (67N, 70R, 219E/Q). The effects of a specific profile on virologic outcome were highlighted by several presentations at the conference.

Antinori and colleagues (Abstract 709) presented results of a retrospective analysis documenting the effect of TAM1 and K65R mutations on virologic outcome in 172 patients from 10 Italian centers initiating salvage therapy with tenofovir/stavudine. Of these, 88.4% and 77.3% of patients were previously exposed to zidovudine and stavudine, respectively. Virologic failure was defined as an HIV-1 plasma RNA level above 500 copies/mL. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.26 \log_{10} copies/mL and 229 cells/ μ L, respectively. At baseline, 19.1% of patients had TAM1, 10.3% TAM2, 76% M184V, 42% T215Y/E, 33% M41L, 29% D67N, and 22% L210W mutations. At month 12, 50% and 66% of patients achieved plasma HIV-1 RNA levels of below 50 copies/mL and below 500 copies/mL, respectively. Multivariate analysis revealed that presence of either 1 TAM1 or all TAM1 mutations was associ-

ated with a greater risk of virologic failure (adjusted HRs [AHRs], 1.65, $P = .003$ and 2.53; $P = .048$, respectively). M184V was associated with a greater reduction in plasma HIV-1 RNA level at month 6 and 12, AHR for virologic failure of 0.36 ($P = .024$) in multivariate analysis. Among 17 evaluable patients with virologic failure, no K65R was detected at baseline or month 12; the prevalence of stavudine associated mutations increased from baseline: D67N from 23% to 46%; L210W from 31% to 67%; K219Q from 7.7% to 15%. The authors concluded that accumulation of TAMs, not K65R, is most predictive of virologic failure on tenofovir/stavudine.

Landman and colleagues (Abstract 710) presented data on the virologic outcome of salvage antiretroviral therapy in the presence of the K65R mutation. Fourteen patients from the TONUS trial (antiretroviral-naïve patients on tenofovir/lamivudine/abacavir) and 8 patients from GS 903 study (randomized study of antiretroviral-naïve patients on tenofovir/lamivudine/efavirenz or stavudine/lamivudine/efavirenz) were included. Baseline mutations included K65R/M184V ($n = 14$), K65R/M184V/NNRTI mutant ($n = 5$) and K65R/NNRTI mutant ($n = 3$). The median baseline plasma HIV-1 RNA level was 6336 copies/mL. Salvage therapy was initiated: tenofovir and lamivudine were continued in 5 and 9 patients, respectively; zidovudine and didanosine were started in 13 and 11 patients, respectively; a PI was the third agent in 16 patients and an NNRTI in 6 patients. At week 48, 86% achieved a plasma HIV-1 RNA below 50 copies/mL and the mean replicative capacity was 54%. The K65R/M184V double mutants were hypersusceptible to efavirenz, nevirapine, and zidovudine. The authors concluded that this may have contributed to the virologic response in patients receiving efavirenz or zidovudine as part of salvage therapy. Johnson and colleagues (Abstract 711) studied the effect of the number of baseline PI mutations on virologic response at week 96 in a post-hoc analysis of the BMS AI424-045 study. Subjects ($n = 358$) were randomized to 1 of 3 arms: atazanavir 300 mg once daily/ritonavir 100 mg once daily ($n = 120$); lopinavir 400 mg twice daily/ritonavir 100 mg twice daily ($n = 123$); and atazanavir 400 mg once daily/saquinavir 1200 mg once daily ($n = 115$). All patients received

tenofovir and an nRTI. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.44 \log_{10} copies/mL and 295 cells/ μ L, respectively. Median number of baseline PI and nRTI mutations were 2 and 3, respectively. In 33% of patients in the atazanavir arms and in 38% in the lopinavir arm, 4 or more PI mutations were present at baseline; among these, the mean decreases in plasma HIV-1 RNA levels at week 96 were 1.71 \log_{10} copies/mL, 0.93 \log_{10} copies/mL, 1.81 \log_{10} copies/mL in the atazanavir/ritonavir, atazanavir/saquinavir, and lopinavir/ritonavir arms, respectively. Among individuals with fewer than 4 PI mutations at baseline, the mean decreases were 2.47 \log_{10} copies/mL for atazanavir/ritonavir; 2.17 \log_{10} copies/mL for atazanavir/saquinavir, and 2.21 \log_{10} copies/mL for lopinavir/ritonavir. The atazanavir/saquinavir arm was the least effective virologically, irrespective of the number of PI mutations.

Pharmacology

Selected Drug-Drug Interactions

Enteric-coated Didanosine/Atazanavir. Enteric coated didanosine (didanosine EC) and atazanavir are potential components of a once-daily antiretroviral regimen. However, didanosine is usually given without food and atazanavir with food. Kaul and colleagues examined the interaction of these 2 drugs when given together with food in 35 healthy volunteers (Abstract 648). They found that the C_{max} and area under the concentration curve (AUC) of didanosine were reduced approximately 35% when given with food and either atazanavir 400 mg or atazanavir 300 mg/ritonavir 100 mg compared with being given in the fasted state. The pharmacokinetics of atazanavir were not affected by coadministration of didanosine.

Buprenorphine and Efavirenz. Buprenorphine is a partial opioid agonist that was recently approved by the US Food and Drug Administration (FDA) for the treatment of opioid dependence. Efavirenz lowers the plasma levels of methadone, potentially leading to a withdrawal syndrome. The effects of efavirenz on buprenorphine pharmacokinetics are unknown. McCance-Katz and colleagues studied buprenorphine pharmacokinetics

before and after 1 week of efavirenz administration in 10 buprenorphine-maintained, HIV-seronegative volunteers (Abstract 653). They found that efavirenz lowered the exposure (ie, AUC) by 50% and increased the rate of buprenorphine clearance. However, efavirenz did not cause opioid withdrawal in these volunteers; the efavirenz levels were in the therapeutic range. This suggests that buprenorphine may be preferable to methadone in HIV-infected patients who are prescribed efavirenz.

Rifampin and Atazanavir. Burger and colleagues examined the interaction of rifampin and atazanavir in HIV-seronegative volunteers (Abstract 657). Rifampin reduces the levels of other PIs and they should not be coadministered. The investigators determined the atazanavir pharmacokinetics using 3 different once-daily doses of atazanavir/ritonavir (300 mg/100 mg, 300 mg/200 mg, 400 mg/200 mg) given with rifampin 600 mg once daily. They compared these doses with atazanavir given without rifampin (400 mg without ritonavir and 300 mg with ritonavir 100 mg). They found that all 3 doses given with rifampin yielded atazanavir levels significantly lower than levels seen with 300 mg/100 mg once-daily. Compared with atazanavir 400 mg once daily, only atazanavir/ritonavir 400 mg/200 mg yielded comparable 24-hour AUC and C_{max} values. However, all 3 doses given with rifampin yielded significantly lower minimum concentration (C_{min}). The authors concluded that atazanavir should not be co-administered with rifampin at the doses studied.

Omeprazole and Atazanavir. Proton pump inhibitors are known to lower levels of atazanavir. Agarwala and colleagues tried 2 different strategies to overcome this interaction in HIV-infected volunteers ($n = 48$) (Abstract 658). They first assessed atazanavir pharmacokinetics after giving atazanavir/ritonavir 300 mg/100 mg once daily for 10 days. They divided the cohort into 3 groups who all received omeprazole 40 mg once daily. Group A had no other change, Group B took the medications with 8 ounces of cola, and Group C increased their atazanavir dose to 400 mg/100 mg once daily. All 3 groups had atazanavir AUC, C_{max} , and C_{min} levels that were reduced

by 56% to 79% compared with those without omeprazole. The authors concluded that atazanavir should not be given with proton pump inhibitors. Future studies will determine if similar interactions exist with H₂-blockers and antacids.

Antiretrovirals and DMPA. Little is known about the interaction of depo-medroxyprogesterone acetate (DMPA) and antiretrovirals. Cohn and colleagues presented data from A5093, an open-label nonrandomized study of the effect of DMPA on the pharmacokinetics of selected PI and NNRTI therapies in HIV-infected women (Abstract 82). The participants were taking 2 nRTIs plus nevirapine (n = 13), efavirenz (n = 14), or nelfinavir (n = 20). Participants were followed up for 12 weeks after receiving an injection of DMPA. According to progesterone levels, none of the women ovulated during the study supporting the efficacy of DMPA. The AUCs for efavirenz, nelfinavir and M8 (the active metabolite of nelfinavir) were not affected by DMPA. There was a statistically, but not clinically, significant increase in the AUC of nevirapine. The DMPA was well tolerated and seems to be safe and effective in women taking these antiretrovirals.

Pharmacogenetics

CYP2B6 and Efavirenz. Investigators from the ACTG 5095 study have previously reported a relationship between genetic polymorphisms in the CYP2B6 gene and efavirenz pharmacokinetics. They found that the G516T change, especially being homozygous for that allele (T/T), was associated with delayed clearance of efavirenz and increased central nervous system side effects. Haas and colleagues extended this observation to speculate how this polymorphism would lead to selective pressure for the development of NNRTI resistance should efavirenz be stopped (Abstract 651). Observed data from ACTG 5095 study subjects showed that the half-life of efavirenz was 23, 28 and 53 hours among G/G, G/T, and T/T subjects. Based on this, they calculated that efavirenz concentrations would be above the IC₅₀ in these subjects for 7, 9, and 19 days. People with the T/T alleles at position 516 in the CYP2B6 are predicted to be at the highest risk for developing NNRTI resistance when stopping efavirenz.

R-Novoa and colleagues confirmed these observations concerning the CYP2B6 polymorphisms in a separate cohort (Abstract 652). Among 111 white patients starting efavirenz, 49% had G/G, 44% had G/T, and 7% had T/T alleles. The T/T and G/T allele-patients had higher plasma levels, and 40% and 19% had “toxic” levels (> 4 µg/mL) compared to 0% with the G/G alleles. Twenty percent of G/G patients had subtherapeutic levels (< 1 µg/mL). Central nervous system symptoms were more common among T/T and G/T patients. The risk of virologic failure was not related to the polymorphism but follow-up was limited to 12 weeks. The authors concluded that genetic testing for the G516T polymorphism may be useful for tailoring efavirenz therapy. Haas and colleagues also found that the CYP2B6 G516T polymorphism was associated with higher efavirenz levels among subjects in ACTG 384 (Abstract 81). However, it was not related to subsequent virologic outcomes with 3 years of follow-up.

P-glycoprotein. P-glycoprotein and multidrug resistance protein-1 are cellular efflux transporters that have the potential to lower intracellular levels of antiretroviral medications. Chandler and colleagues examined the correlation of these transporters and the expression of CXCR4 in PBMCs (Abstract 665). They found that both of these transporters were significantly correlated with expression of CXCR4 when examining PBMCs as a whole and with CD4+ cells in particular. These findings suggest that high expression of CXCR4 in PBMCs and CD4+ cells may make HIV infection more difficult to treat. Hartkoorn and colleagues found that rifampin upregulates P-glycoprotein expression, but not multi-drug resistance protein-1 (Abstract 666). They also found that rifampin is a substrate for both of these efflux transporters. This study suggests that rifampin could lower intracellular levels of antiretrovirals by promoting expression of efflux transporters.

Hulgan and colleagues assessed P-glycoprotein activity in samples derived from a large clinical cohort (Abstract 667). P-glycoprotein activity was higher in naive CD4+ lymphocytes than in total CD4+ lymphocytes. P-glycoprotein activity was also higher among African Americans than among whites, and in women than among men.

Therapeutic Drug Monitoring

Researchers from the California Collaborative Treatment Group (CCTG) enrolled 199 patients into a therapeutic drug monitoring trial (CCTG 578). Patients were starting a new PI or NNRTI-based antiretroviral regimen (Abstract 640). After 2 weeks, blood levels of PIs or NNRTIs were measured. A panel of experts reviewed the drug levels along with treatment history, CD4+ cell count, plasma HIV-1 RNA level and clinical toxicities, to arrive at a recommendation to either change the PI or NNRTI dose, or to leave it the same. Patients were randomized to receive the recommendation or to remain on the same dose. The mean baseline CD4+ count was 189 cells/µL and the mean plasma HIV-1 RNA was 5.2 log₁₀ copies/mL. The median age was 40 years. Twenty-nine percent were starting their first antiretroviral regimen.

Overall, the expert panel recommended changing the PI or NNRTI dose in 67 patients (38%). All but 3 recommendations were to increase the dose. The factors associated with needing to increase the dose were having a higher weight or body mass index, use of either efavirenz or lopinavir/ritonavir, and being non-Hispanic. In the adjusted analysis, weight and use of either efavirenz or lopinavir/ritonavir remained associated with a dose-adjustment recommendation. Interestingly, adherence, age, and sex were not associated. The main result of this study, whether dose adjustment improves virologic outcomes, has not been analyzed yet.

Nettles and colleagues did intensive blood sampling on 10 highly adherent patients (Abstract 642). They drew blood 3 times a week for at least 3 months to assess the frequency of “blips” in the plasma HIV-1 RNA level. They also drew plasma levels of the NNRTI or PI that the patient was taking. Low plasma levels did not coincide with the blips. The drug levels in a given individual varied by as much as 43% for PIs and 26% for NNRTIs. This indicates that assessment of numerous drug levels may be necessary before making any treatment decisions based on this information.

Mother-to-Child Transmission of HIV

Single-dose nevirapine received significant attention at this year's conference

as new data became available from several ongoing clinical trials in sub-Saharan Africa focusing on issues of resistance and treatment alternatives. Fueled by recent public controversy surrounding the conduct of the HIVNET 012 trial, the use of single-dose nevirapine has been highly scrutinized despite significant successes in decreasing the rates of mother to child transmission of HIV (MTCT). Half a million doses of nevirapine have been distributed worldwide since the results of the HIVNET 012 study demonstrated a 40% reduction in MTCT (Abstract 8). Data presented at this year's conference shed new light on the ongoing controversy surrounding the efficacy and the implications of resistance following single-dose nevirapine.

Towne-Gold and colleagues (Abstract 785) presented results from a study evaluating efficacy of 1 NNRTI with 2 nRTIs administered during pregnancy. In the MTCT-Plus program in Cote d'Ivoire, 205 women were enrolled; 88 of them with a median CD4+ count of 185 cells/ μ L received a regimen of zidovudine/lamivudine/nevirapine starting at 26 weeks; 114 women with median CD4+ count of 472 cells/ μ L received MTCT prophylaxis with zidovudine/lamivudine starting at 32 weeks until 3 days postpartum followed by an intrapartum single-dose nevirapine. Infants received 1 week of zidovudine and single-dose nevirapine on day 3 after birth. Infant plasma HIV-1 RNA was assessed at 4 and 6 weeks. Among infants of women receiving zidovudine/lamivudine/nevirapine, 69 of 80 live births tested to date revealed 1 infection in the setting of maternal nonadherence, giving an overall transmission rate of 1.45% (95% CI, 0.00-7.8) at 4 weeks. Among infants born to women receiving the MTCT regimen, 77 of 94 live births were tested, 3 infants were HIV infected (rate, 3.89%; 95% CI, 0.03-9.67) whose mothers had only taken single-dose nevirapine at delivery. Six mothers in the zidovudine/lamivudine/nevirapine group experienced grade 3 adverse events (rash, n=6; hepatotoxicity, n=1) requiring drug switch. The investigators note that these low rates of transmission of HIV-1 in mothers on potent antiretroviral therapy are similar to those seen in studies from high resource settings. Based on these preliminary data, they conclude that potent antiretroviral therapy during

pregnancy can dramatically reduce the risk of MTCT in women with advanced disease in African populations. Several smaller studies drew similar conclusions regarding the efficacy of combination prophylactic therapy antenatally to prevent MTCT.

Jourdain and colleagues (Abstract 782) evaluated 137 HIV-1 infected pregnant women in Thailand receiving minimal or no antenatal care who were given emergency antiretroviral prophylaxis with zidovudine antenatally where possible followed by intrapartum single-dose nevirapine. Infants were given single-dose nevirapine and 4 weeks zidovudine after birth. Ninety women (66%) did not receive any zidovudine before labor and the remainder received the drug for less than 15 days. 95% of infants received zidovudine for 6 weeks. Overall transmission rates were 15.7% among 103 newborns in which single-dose nevirapine was administered to both the mother and infant and 23.2% in 29 cases in which only the newborn received nevirapine. The investigators concluded that compared with their recently published results of the Program for HIV Prevention and Treatment (PHPT)-2 study where women received zidovudine from 28 weeks gestation, the efficacy of peripartum single-dose nevirapine in this cohort was poor, emphasizing the need for extended combination therapy in the entire third trimester of pregnancy.

Tubiana and colleagues (Abstract 810) presented results from an observational, single-center study evaluating the safety and efficacy of indinavir/ritonavir in 32 French women. Twenty-one antiretroviral-experienced and 11 -naive pregnant women received indinavir/ritonavir (400 mg/100 mg bid) in combination with 2 nRTIs. The most common nRTI regimens were zidovudine/lamivudine (91%), stavudine/lamivudine (6%), and zidovudine/didanosine (3%). All women received intrapartum zidovudine infusion and infants received 6 weeks of zidovudine. The median exposure to indinavir/ritonavir was 24 weeks, with 87% completing their pregnancy on the regimen. Overall, by ITT analysis, 91% of women at delivery had plasma HIV-1 RNA levels below 400 copies/mL with median CD4 count of 352 cells/ μ L. Thirty-three live births were reported with 1 spontaneous miscarriage and 2 twin births; none were infected at 0, 3,

and 6 months as assessed by HIV RNA and DNA PCR. No renal toxicity or hyperbilirubinemia was reported in any of the 33 infants. Four women discontinued indinavir/ritonavir prior to delivery, 1 for virologic failure and the remainder for intolerance (2 with xerosis) and 1 with mild liver enzyme elevation). The investigators concluded that a boosted PI regimen may improve adherence, decrease MTCT, and preserve future therapeutic options for the mother, indicating that a larger prospective study is warranted.

Ngo-Giang-Huong and colleagues (Abstract 802) found decreased rates of nevirapine resistance among perinatally infected, nevirapine-exposed infants treated with zidovudine. Mothers in this subset of the PHPT-2 cohort from Thailand received single-dose nevirapine plus varying durations of prenatal zidovudine prophylaxis. Overall resistance rate in infants given single-dose nevirapine or placebo plus zidovudine for 1 to 6 weeks was 8%, lower than that described for most studies evaluating exposure to single-dose nevirapine alone.

The DREAM Study

Palombi and colleagues (Abstract 67) presented data evaluating the efficacy and safety of antiretroviral therapy among HIV-1-infected Mozambique women enrolled in the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) study. A total of 778 pregnant women received nevirapine with either zidovudine/lamivudine or stavudine/lamivudine, starting at 25 weeks and continued to month 6 postpartum. The infants were not treated after birth. Women with CD4+ cell counts above 200 cells/ μ L stopped therapy 1 month post delivery. The baseline CD4+ count and plasma HIV-1 RNA level were 498 cells/ μ L and 4.15 log₁₀ copies/mL, respectively. The median duration of antiretroviral therapy prior to delivery was 74 days; 65 women were lost to follow-up. The authors found that, by ITT analysis, the cumulative transmission rate of HIV was 6.1% and 1.4% respectively, among infants breastfed in the first month. Factors associated with transmission were pre-antiretroviral therapy plasma HIV-1 RNA level, antenatal duration of therapy, presence of a sexually transmitted disease, and non-adherence to or discontinuation of treat-

ment. There were 42 samples available for genotypic testing 6 months after suspension of treatment; all were clade C virus and 88% had no evidence of resistance. K103N and G190S mutations were seen in 1 and 5 samples, respectively. Grade 3 or 4 hepatotoxicity occurred in 6% of women but there were no nevirapine related deaths. The investigators concluded that 3-drug antiretroviral therapy is effective, well tolerated and, compared to single-dose nevirapine, associated with lower rates of resistance and HIV transmission.

ANRS DITRAME Plus

Chaix and colleagues (Abstract 72 LB) described the resistance rates in the ANRS DITRAME Plus study. This was an open-label study enrolling 329 women receiving zidovudine/lamivudine starting at week 32, an additional intrapartum dose of zidovudine/lamivudine with single-dose nevirapine, and then 3 days of zidovudine/lamivudine. Infants received 1 week of zidovudine, and single-dose nevirapine on day 2. Six weeks after birth, the rate of MTCT was 4.7%, and transmission was associated with higher viral load and lower CD4+ cell count in the mothers. Genotypic analysis performed at baseline and 4 weeks postpartum on 16 transmitting mother/infant pairs and 80 non-transmitting mothers/infant pairs showed 1.14% and 8.3% rates of nevirapine and lamivudine resistance, respectively. Among the 16 HIV-infected infants, 1 developed K103N/Y181C and M184V mutations, and 3 had M184V despite never receiving lamivudine. Among the nontransmitting women, 1 had both K103N and M184V and 7 had M184V alone. Multivariate analysis demonstrated that the duration of zidovudine/lamivudine therapy was associated with development of M184V. The authors concluded that this regimen may reduce the rates of drug resistance seen after single-dose nevirapine.

MASHI

The resistance data from the MASHI trial in Botswana presented by Shapiro and colleagues (abstract 74LB) suggested that combination antiretroviral therapy may not always be effective at preventing the emergence of nevirapine resistance. This randomized, partially blinded,

placebo-controlled study enrolled 1200 HIV-infected pregnant women to receive nevirapine at delivery with nevirapine for the infant, or placebo at delivery and placebo for the infant. All women and infants received 1 month of zidovudine. The study design was revised after 17 months to administer nevirapine to every infant at birth. Mother/infant pairs were further randomized to formula feed or breastfeed with 6 months of infant zidovudine prophylaxis. Antiretrovirals became available to women with AIDS in the second study period and were initiated by 71 of 694 women. This was initially designed as a superiority trial of maternal/infant nevirapine (N/N) versus maternal/infant placebo (P/P), but the revised study evaluated equivalence of P/N to N/N. Results of the 2 study periods were evaluated separately. Transmission rates in the first study period were higher in the P/P group (4.5% vs. 3.8% in the N/N group) and rose after 1 month (6.2% vs. 5.3% respectively, $P = .7$). Transmission rates in the second period were 3.8% versus 2.3% in those receiving placebo and nevirapine respectively, rising to 4.3% and 3.7% ($P = .70$), respectively, at 1 month after birth, meeting predetermined criteria for equivalence in the 2 arms. Transmission rates overall for both study periods were 4.0% at birth and 4.7% at 1 month. Analysis of feeding strategy favored the nevirapine arm in the first period, but not in the revised study. Resistance was found in 44% (69/157) of nevirapine exposed women tested at 1 month postpartum and 33% harbored the nevirapine associated K103N mutation. The investigators concluded that results from the initial period demonstrate nonsuperiority of nevirapine as background to zidovudine therapy, with a possible benefit seen in formula-fed infants. Likewise, results from the revised study period showed no advantage of adding maternal nevirapine in the context of infant nevirapine. Coupled with high rates of nevirapine resistance, they further conclude that nevirapine sparing strategies should be considered.

A further analysis of this cohort evaluating effect of feeding strategy on infant mortality and transmission rates was presented by Thior and colleagues (abstract 75LB). The 1179 infants in the MASHI trial were randomized as described above to either formula feed-

ing with 1 month zidovudine or breast feeding during 6 months of infant zidovudine prophylaxis. Primary outcomes were cumulative rate of HIV infection at 7 months and 18-month HIV-free survival. As noted above, in the initial study design, HIV infection rates strongly favored the formula-fed infants in the nevirapine arm, but this advantage decreased in the revised study. Overall, cumulative HIV infection rates were slightly better the formula-fed arm (5.6% vs. 9.1%, $P = .04$) at 7 months although mortality at 7 months was higher in this group (9.3% vs. 4.9%; $P = .0003$). HIV-free survival was similar in both groups (86% vs. 84% for the formula-fed arm and breast-fed and zidovudine arm, respectively). Overall, the investigators concluded that both formula feeding and breast feeding/zidovudine are reasonable strategies in this population.

Standard genotype assays may miss mutations comprising less than 20% of the viral population. The utility of highly sensitive resistance testing following single-dose nevirapine was highlighted by 3 presentations at the conference.

Johnson and colleagues (Abstract 100) used real-time reverse transcriptase polymerase chain reaction (RT-PCR) to identify low-frequency mutations among pre- and postpartum samples from women enrolled in a South African MTCT study receiving single-dose nevirapine at labor. The nevirapine-associated K103N mutation was not detected by conventional sequencing methods in any of the pre-nevirapine specimens was detected in and 10 of 50 of specimens (20%) collected at 6 weeks to 36 weeks postpartum. Of the 40 specimen with no resistance mutations by conventional assays, the K103N was detected by the RT-PCR method in an additional 16 specimens (40%). Five samples had the Y181C mutation by RT-PCR analysis that was not detected by conventional sequencing. Clonal sequencing analysis of 5 positive samples confirmed the presence of detected mutations in all samples, with frequencies of 1.1% to 11% of total virus population. Thus by RT-PCR, overall resistance estimates in this cohort were revised upward to 65%, representing a 62% increase in incidence of nevirapine resistance compared with population-based sequencing detection methods.

Similar findings were presented by Palmer and colleagues (Abstract 101),

who used an allele specific RT-PCR with primers developed to detect NNRTI resistance associated mutations, K103N and Y181C. Investigators evaluated samples from 29 women from the South African MTCT trial who received single-dose nevirapine at the onset of labor. By standard genotypic analysis, 8 (27%) had NNRTI resistance at 6 weeks and 6 months post delivery but not at 12 months; 11 had resistance mutations at 6 weeks only, and 10 demonstrated no resistance at any time point. Using allele specific RT-PCR they were able to quantify resistance mutations in these samples at below 0.1% frequency. At 12 months after nevirapine exposure, resistance mutations were detected in 7 of 8 (88%) in group 1, with a median frequency of 3.2%. Similarly, resistance was detected in 100% of group 1, 80% of group 2, and 50% of group 3 samples at 6 months, with frequencies ranging from 0.9% to 10%. Extrapolation of these results to the total cohort in this trial indicates that 69% of women receiving single-dose nevirapine could have NNRTI resistance mutations at 4 months, 32% at 6 months, and 22% at 12 months. Thus the incidence of NNRTI resistance was double that reported by standard genotyping.

Loubser and colleagues (Abstract 102) reinforced these findings in a similar study assessing the frequency of the K103N mutation in 18 postpartum samples from women receiving single-dose nevirapine. Resistance mutations demonstrated in 50% of samples at 6 weeks postpartum by standard detection methods increased in prevalence to 89% by the RT-PCR method. Longitudinal follow-up of 16 women demonstrated that these mutations faded over time, detected in only 25% at 1 year.

Taken together, these 3 studies of RT-PCR detection of nevirapine resistance mutations in HIV-1 seropositive African women with subtype C virus indicate that nevirapine resistance is likely present in the majority of women following single-dose nevirapine exposure and resistance rates are highly underestimated by standard population-based sequencing. The frequency of resistance mutations declines over time but can remain above pretreatment levels for at least 1 year. Despite this evidence, RT-PCR remains cost prohibitive for widespread use in resource poor settings, yet these data

emphasize the importance of assessing the clinical implications of resistant variants and should be employed in future clinical research studies.

Despite the mounting concerns about single-dose nevirapine for prevention of MTCT of HIV, data presented by Martinson and colleagues (Abstract 103) suggest that the use of single-dose nevirapine in primigravid women does not necessarily limit its utility in subsequent pregnancies. Investigators in Soweto enrolled 318 infant/mother pairs from 13 prenatal clinics in this pilot case-controlled study with a primary aim to compare rates of MTCT of HIV in women exposed to single-dose nevirapine in 2 successive pregnancies by evaluating maternal viral load, CD4+ count, and HIV resistance prior to single-dose nevirapine and 6 weeks postpartum. Infection in infants was determined by PCR at 6 weeks. Preliminary results presented on 77 of 106 mother-infant pairs with prior nevirapine exposure and 140 nevirapine-naïve matched controls demonstrated that although transmission rates were higher in the cohort receiving single-dose nevirapine for the second time (10.7% vs. 3.0%), the difference was not statistically significant. These rates are comparable to that observed in the general population exposed to single-dose nevirapine in a single pregnancy, leading investigators to conclude that the efficacy of single-dose nevirapine in second pregnancies is reasonably maintained, and in the setting of transmission rates of 25% in the absence of prophylaxis, single-dose nevirapine remains a significantly beneficial modality for prevention of MTCT of HIV. Resistance rates were comparable in the 2 groups. Implications of these results will be more apparent as results of the entire study population become available.

Eshelman and colleagues (Abstract 799) reported that resistance rates among African women given single-dose nevirapine may be clade specific. They found resistance rates as high as 67% (45/67) in Malawian women following single-dose nevirapine for MTCT in women with subtype C virus subjects from the NVAZ trial compared with 36% (28/147) of patients with subtype D and 19% (35/98) of patients with subtype A virus from Ugandan subjects from the HIVNET 012 trial. The proportion of women with 2 or more mutations asso-

ciated with nevirapine resistance was also higher in women with subtype C virus (43%) than women with subtype D (16%) or A (8%). The most common mutations were K103N and Y181C. Multivariate analysis of risk factors associated with development of nevirapine resistance in this study demonstrated maternal viral load at delivery and viral subtype were independent predictors of nevirapine resistance (C vs A: OR, 8.38; 95% CI, 4.19-16.76. C vs D: OR, 3.27; 95% CI, 1.53-3.25), but age, parity, and nevirapine dosing time relative to delivery were not. The authors cautioned that as subjects in this study were taken from different cohorts (subtype C patients from NVAZ trial and subtype A and D from HIVNET 012 trial) other factors may contribute to the observed differences in nevirapine resistance. However, these intriguing data indicate that further investigation of subtype dependent differences in nevirapine resistance rates is warranted.

SIMBA

Data on rates of drug resistance among breast-fed infants in the Ugandan SIMBA trial were presented by Giuliano and colleagues (Abstract 99). The SIMBA trial evaluated safety and efficacy of lamivudine and nevirapine in preventing MTCT of HIV in 404 infants of infected mothers receiving didanosine/zidovudine therapy from 36 weeks gestation to 1 week postpartum. Infants were randomized to receive either daily nevirapine or lamivudine throughout breastfeeding or until HIV infection was confirmed. Of the 30 infants who were infected during the study period, 26 were evaluated for resistance mutations in this substudy, half received nevirapine, and the other half lamivudine. There were no differences between the 2 arms in preventing transmission. Genotypic analysis demonstrated the lamivudine associated resistance mutation M184V in 9 of 13 (69%) of those in the lamivudine arm initially, yet this faded over time and was not present in samples collected 3 to 6 months later. Twelve of the 13 (92%) infants in the nevirapine arm had nevirapine resistance mutations, 9 with Y181C and 3 with multiple mutations; all persisted on follow up after discontinuation of prophylaxis. Two mothers in the lamivudine arm had K103N and

M41L mutations on enrollment and at delivery and these were demonstrated in their infants' samples. Two mothers in the nevirapine group had mutations G190A and V108I at baseline, but only the latter persisted in their infants' samples. No zidovudine/didanosine associated mutations were observed in the 22 mothers samples tested at delivery. The investigators pointed out that while the study numbers are limited, postpartum prophylaxis with either nevirapine or lamivudine invariably led to selection of resistance mutations in infected infants in this study, especially in the nevirapine arm. These results should be considered in development of prophylaxis regimens to be studied in future clinical trials.

Conclusions

The 2005 CROI solidified its status as the premier conference of the year providing state-of-the-art information on antiretroviral therapy from completed and ongoing studies and introducing new avenues of investigation. Presentations covered the range of important advances in HIV care and research, in particular the recent advances in new antiretroviral agents, experience with

therapy in the international setting, and the debate about the use of single-dose nevirapine in developing countries.

Financial Disclosures: Dr Sobieszczyk has no affiliations with commercial organizations that may have interests related to the content of this article. Dr Wilkin has received grant and research support or honoraria from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, and Tibotec. Dr Talley has no affiliations with commercial organizations that may have interests related to the content of this article. Dr Hammer has served as a consultant to Boehringer-Ingelheim, Pfizer, Progenics, and Tibotec.

Additional Suggested Reading

De Jesus E, Elion R, Cohen C, et al. Week 24 analysis of once-daily (QD) trizivir (TZV) and tenofovir DF (TDF) in antiretroviral naive subjects (COL40263). [Abstract H-564.] 44th Interscience Conference on Antimicrobial Agents and Chemotherapy. October 30-November 2, 2004; Washington, DC.

Demarest J, Bonny T, Vavro C, et al. HIV-1 co-receptor tropism in treatment naive and experienced subjects. [Abstract H-1136.] 44th Inter-

science Conference on Antimicrobial Agents and Chemotherapy. October 30-November 2, 2004; Washington, DC.

Lu J, Sista P, Giguel F, Greenberg M, Kuritzkes DR. Relative replicative fitness of human immunodeficiency virus type 1 mutants resistant to enfuvirtide (T-20). *J Virol.* 2005;78:4628-4637.

Moyle G, Nelson M, Higgs C, et al. A randomised open label comparative study of combivir + efavirenz (2 class triple therapy) versus trizivir + tenofovir (single class quadruple therapy) in initial therapy for HIV-1 infection. [Abstract H-1131.] 44th Interscience Conference on Antimicrobial Agents and Chemotherapy. October 30-November 2, 2004; Washington, DC.

Rizzardi GP, Harari A, Capiluppi B, et al. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. *J Clin Invest.* 2002;109:681-688.

A list of all cited abstracts appears on pages 45 to 50.

Top HIV Med. 2005;13(1):24-44
Copyright 2005, International AIDS Society–USA