

Advances in Antiretroviral Therapy

Magdalena E. Sobieszczyk, MD, Eoin P.G. Coakley, MD, Timothy J. Wilkin, MD, MPH, and Scott M. Hammer, MD

Antiretroviral therapy was a focus of many of the studies reported at the 11th CROI. This year, data on new drugs, refinements in the management of treatment-naive and treatment-experienced persons, the impact of drug resistance (particularly following exposure to a single dose of nevirapine), and the growing experience with antiretrovirals in the developing world were the dominant themes. This review summarizes new information relevant to clinicians and clinical researchers.

Investigational Antiretroviral Drugs

Results of select studies of investigational antiretroviral drugs are summarized in Table 1.

Entry Inhibitors

CCR5 Antagonists. SCH D is a CCR5 receptor antagonist that has supplanted SCH C in the development process. SCH D proved more potent in vitro than SCH C and also has a longer half-life, better absorption, and higher bioavailability, based on rat and monkey studies. Schurmann and colleagues (Abstract 140LB) presented results of a study evaluating SCH D monotherapy in chronically HIV-infected subjects. A total of 48 subjects with baseline plasma HIV-1 RNA levels between 5,000 copies/mL and 200,000 copies/mL and CD4+ counts greater than 250 cells/ μ L, who were off antiretroviral therapy for 8 weeks, were randomized to receive 10 mg of SCH D twice-daily (bid); 25 mg of

SCH D bid; 50 mg of SCH D bid, or placebo for 14 days. Mean change in plasma HIV-1 RNA levels from baseline was $-1.08 \log_{10}$ copies/mL, $-1.56 \log_{10}$ copies/mL, and $-1.62 \log_{10}$ copies/mL in the 10 mg-, 25 mg-, and 50 mg-dose groups, respectively. All doses were well tolerated. One patient with plasma HIV-1 RNA reduction of greater than 1.5 \log_{10} copies/mL had evidence of a transient switch to X4 virus after treatment.

GW873140 is a novel, orally bioavailable CCR5 receptor antagonist. Demarest and colleagues (Abstract 139) presented data from a double-blind, randomized, placebo-controlled, single- and multiple-dose escalation study conducted in 70 non-HIV infected volunteers. Preliminary data indicated that this compound was well tolerated with no serious adverse events. Most of the side effects were gastrointestinal; 4 patients who received multiple doses had lipase increases. Ingesting the drug with food increased the area under the concentration curve (AUC) and C_{max} by means of 1.7- and 2.2-fold, respectively. CCR5 receptor occupancy depended on the drug dose and the sampling time; baseline binding analyses showed 0% receptor occupancy, 93% to 99% dose-dependent occupancy at 2 hours, and over 97% receptor occupancy at 12 hours, at all doses. Receptor-binding analyses 24 hours after a single dose showed median receptor occupancy ranging from 68% to 88%, despite compound concentrations below or near assay limits. At 2 and 12 hours after multiple dosing with GW873140, the CCR5 occupancy was greater than 97%. These findings suggest a long half-life for the compound-receptor binding, with once daily (qd) or bid dosing

schedules. Studies of GW873140 in HIV-infected individuals are planned.

Attachment Inhibitors. BMS-488043 is an orally available HIV entry inhibitor, which binds specifically to gp120 and interrupts the attachment of the viral envelope to the CD4 receptor. Lin and colleagues (Abstract 534) demonstrated that BMS-488043 binds reversibly to gp120 with a 1:1 stoichiometry, and that its activity is coreceptor independent. Data from a limited number of isolates indicate that this agent is effective against both X4 and R5 laboratory strains, and has good in vitro potency against subtype B clinical isolates (median 50% effective concentration [EC_{50}] of 37 nM). BMS-488043 has a long half-life and a good safety profile in non-infected volunteers in single-dose and in multiple-dose studies (Abstract 535). C_{max} and AUC values appeared to be dose-related for those of 200 mg to 800 mg, with no significant increase in exposure seen at higher doses. Exposures were generally higher with a high-fat meal than with a light meal. The authors concluded that a dose of 800 mg bid is expected to provide adequate plasma concentrations for suppression of subtype-B HIV-1 isolates.

Hanna and colleagues (Abstract 141) compared the antiviral activity of BMS-488043 monotherapy with placebo over 8 days. HIV-1-infected patients with plasma HIV-1 RNA levels between 5,000 and 500,000 copies/mL and CD4+ counts above 250 cells/ μ L, who were treatment naive or off antiretrovirals for more than 16 weeks, received 800 mg (n = 12) or 1800 mg (n = 12), bid. The mean baseline CD4+ count was 395 cells/ μ L and plasma HIV-1 RNA level was $4.61 \log_{10}$ copies/mL. Approximately 50% of subjects were treatment naive. On day 8, the mean change in plasma HIV-1 RNA level from baseline was $-0.72 \log_{10}$ copies/mL and $-0.96 \log_{10}$ copies/mL for 800 mg and 1800 mg doses, respectively. The majority of patients had at least a 1- \log_{10} decline in

Dr Sobieszczyk is a Post-Doctoral Research Fellow at Columbia University College of Physicians and Surgeons, New York, NY. Dr Coakley is Assistant Professor at Tufts University School of Medicine and Attending Physician in the Division of Geographic Medicine and Infectious Diseases at Tufts-New England Medical Center, Boston, Mass. Dr Wilkin is Instructor of Medicine at Weill Medical College of Cornell University, New York, NY. Dr Hammer is Professor of Medicine at Columbia University College of Physicians and Surgeons and Chief of the Division of Infectious Diseases at Columbia Presbyterian Medical Center, New York, NY.

Table 1. Summary of Selected Investigational Antiretroviral Drugs

Drug	Abstract(s)	Drug Class or Mechanism of Action	Development Stage	Results
D-D4FC	137	nRTI	Phase II studies in HIV-infected individuals	1.77 log ₁₀ copies/mL drop in plasma HIV-1 RNA levels after 10 days of monotherapy ¹
SPD 754	138, 526, 527, 599	nRTI	Phase II studies in HIV-infected individuals. Long-term toxicity studies in animals	1.65 log ₁₀ copies/mL drop in plasma HIV-1 RNA from baseline after 10 days of therapy with a 1200 mg dose. Comparable efficacy in patients with preexisting NAMs
SN1212/1461	532	nRTI	Preclinical	EC ₅₀ , 10-100 nM
Diarylpyrimidines (DAPYs) and Diarylthiazines (DATAs)	528	NNRTI	Preclinical	EC ₅₀ , 0.4-3.0 nM
678248 and 695634 (678248 prodrug)	529	NNRTI	Preclinical	IC ₅₀ 1.8 nM against wild-type, and IC ₅₀ 0.8-6.8 nM against reverse transcriptase mutants
SCH D	140LB	Entry Inhibitor (CCR5)	Phase II studies in HIV-infected subjects	1.08-1.62 log ₁₀ copies/mL drop in plasma HIV-1 RNA levels after 14 days of monotherapy ²
GW873140	139	Entry Inhibitor (CCR5)	Phase I study in HIV-seronegative subjects	Greater than 97% receptor binding 12 hours after dosing
AMD887	539	Entry Inhibitor (CCR5)	Preclinical	EC ₅₀ , 1-10 nM ⁵
KRH-2731 Orally bioavailable	541	Entry Inhibitor (CXCR4)	Preclinical	EC ₅₀ , 1-4.2 nM
BMS-488043	141, 534, 535	Attachment Inhibitor	Phase II study in HIV-infected subjects	0.72-0.96 log ₁₀ copies/mL drop in plasma HIV-1 RNA levels after 8 days of monotherapy ³
PA-457	545	Gag processing inhibitor (inhibits processing of p24 capsid protein)	Preclinical	Inhibits Gag CA-SP1 cleavage site. Does not inhibit the P450 enzyme system (IC ₅₀ , >100 nM)
TMC114	533	Protease inhibitor	Phase IIa study in HIV-infected subjects	1.24-1.50 log ₁₀ copies/mL drop in plasma HIV-1 RNA levels after 14 days as functional monotherapy ⁴

¹Participants were antiretroviral-naive; median baseline plasma HIV-1 RNA level was 4.2 log₁₀ copies/mL. D-D4FC was administered as monotherapy in doses ranging from 50 mg to 200 mg. The greatest efficacy was seen at the 200 mg dose.

²Subjects had baseline plasma HIV-1 RNA levels between 5,000 copies/mL and 200,000 copies/mL and were off antiretroviral therapy for 8 weeks.

³Mean baseline plasma HIV-1 RNA level was 4.6 log₁₀ copies/mL, and subjects were antiretroviral naive (50%) or off antiretrovirals for more than 16 weeks.

⁴Patients were receiving failing regimen and at baseline had 3 major protease inhibitor (PI) mutations. For patients with more than one PI mutation at baseline, the median drop in plasma HIV-1 RNA levels was 1.44 log₁₀ copies/mL.

⁵When AMD887 was combined with AMD070 (a CXCR4 antagonist), viral replication was suppressed in peripheral blood mononuclear cells.

nRTI indicates nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NAM, nRTI-associated resistance mutation; EC₅₀, 50% effective concentration; IC₅₀, 50% inhibitory concentration

plasma HIV-1 RNA level: 58% in the 800 mg group and 67% in the 1800 mg group. After 8 days of therapy, the mean increase in CD4+ count was 106 cells/ μ L in the 800 mg arm, 48 cells/ μ L in the 1800 mg arm, and 30 cells/ μ L in the placebo arm. These differences, however, were not statistically significant. Overall, the drug was well tolerated; fatigue was noted in 4 of 15 subjects and headaches in 2 of 15 in the 800 mg arm. This proof of concept study supports the further development of this novel class of attachment inhibitors that target gp120.

Monoclonal antibody to CD4. TNX-355 is a viral entry inhibitor, an IgG4 monoclonal antibody that targets domain 2 of the CD4 receptor. This specificity of binding allows for inhibition of post-binding viral entry and fusion without causing immunosuppression. Jacobson and colleagues (Abstract 536) presented results from a study of 21 HIV-1-infected individuals with plasma HIV-1 RNA levels greater than 5,000 copies/mL and CD4+ counts between 100 cells/ μ L and 500 cells/ μ L, who were randomized into 1 of 3 treatment arms: 10 mg/kg every 7 days for 10 weeks ($n=9$); 10 mg/kg on the first day followed by 6 mg/kg every 14 days for 6 doses ($n=10$); and 25 mg/kg every 14 days for 5 doses over 8 weeks ($n=3$). One subject was antiretroviral naive. The mean baseline CD4+ count and plasma HIV-1 RNA level were 332 cells/ μ L and 4.78 log₁₀ copies/mL, respectively. Mean reductions in plasma HIV-1 RNA of 1.11 log₁₀ copies/mL and 0.96 log₁₀ copies/mL occurred by week 2 in the 2 highest doses (10 mg/kg and 25 mg/kg). At week 9 (final dosing), plasma HIV-1 RNA levels had returned to baseline; reduced susceptibility to TNX-355 was seen in 16 subjects. CD4+ counts rose transiently and the maximal median elevations above baseline in the 3 treatment groups were 257 cells/ μ L, 198 cells/ μ L, and 103 cells/ μ L. No CD4+ count depletion was noted. Complete and continuous CD4+ cell coating was observed for a minimum of 14 days after the final dose was administered in each study arm. Two subjects were withdrawn, 1 due to new onset seizure and 1 to a protocol violation (illicit drug use); other serious adverse events

included recurrence of depression and transient acute renal failure. A phase II study of this agent in combination with optimized background therapy in antiretroviral-experienced patients is planned.

Protease Inhibitors

TMC114. Peeters and colleagues (Abstract 533) presented the results of a phase IIa, open label, randomized study of TMC114 coadministered with a boosting dose of ritonavir in protease inhibitor (PI)-experienced patients. Patients with no current AIDS defining illnesses, plasma HIV-1 RNA levels above 2000 copies/mL, previous treatment with 2 to 4 PIs for more than 2 months each, virologic failure of the current regimen, and no nonnucleoside reverse transcriptase inhibitor (NNRTI) use in the failing regimen were eligible for study participation. Fifty patients were randomized into 4 study arms: TMC114 300 mg/ritonavir 100 mg bid ($n=13$); TMC114 600 mg/ritonavir 100 mg bid ($n=12$); TMC114 900 mg/ritonavir 100 mg qd ($n=13$); or continuation of current therapy. Background nucleoside reverse transcriptase (nRTI) treatment was not changed. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.26 log₁₀ copies/mL and 305 cells/ μ L, respectively. At baseline, 16 of 35 patients had virus with phenotypic resistance to all PIs (atazanavir not tested); the median baseline number of major PI mutations in all groups was 3, and no significant genotypic and phenotypic differences for PIs were observed between groups. At day 14 of therapy, the median change in plasma HIV-1 RNA from baseline was -1.24 log₁₀ copies/mL in the 300 mg/100 mg arm; -1.25 log₁₀ copies/mL in the 900 mg/100 mg arm; and -1.50 log₁₀ copies/mL in the 600 mg/100 mg arm. The median plasma HIV-1 RNA change from baseline to day 14 in the 900 mg/100 mg and 600 mg/100 mg study arms was significantly greater than in the control arm ($P<0.05$ and <0.001 , respectively). There were no significant differences in antiviral response in subgroups divided according to baseline plasma HIV-1 RNA. For all treated subjects with more than one major PI mutation, the median plasma

HIV-1 RNA change from baseline to day 14 was -1.44 log₁₀ copies/mL (range -0.47 to -2.49 log₁₀ copies/mL); this was statistically significant compared with the control arm ($P<0.05$). In patients with phenotypic resistance to lopinavir, the median plasma HIV-1 RNA decrease from baseline was 1.50 log₁₀ across all treatment groups; patients with a baseline resistance to all PIs had a median plasma HIV-1 RNA decrease of 1.50 log₁₀ copies/mL. A randomized phase IIb trial is under way to evaluate the optimal dose and schedule of TMC114/ritonavir in PI-experienced patients.

Reverse Transcriptase Inhibitors

D-D4FC. Murphy and colleagues (Abstract 137) presented preliminary results of a study evaluating D-D4FC (no generic name for this drug is yet available) monotherapy in antiretroviral-naive patients. D-D4FC is an nRTI that has in vitro activity against HIV-1 isolates resistant to zidovudine, lamivudine, and other nRTIs. It loses activity only in the presence of the Q151M resistance mutation or an amino acid insertion at position 69 of HIV-1 reverse transcriptase. Thirty subjects with CD4+ counts greater than 50 cells/ μ L and plasma HIV-1 RNA levels greater than 5,000 copies/mL were randomized to receive 50 mg, 100 mg, or 200 mg of D-D4FC (qd), or placebo for 8 days. At baseline, the median plasma HIV-1 RNA level and CD4+ count were 4.2 log₁₀ copies/mL and 486 cells/ μ L, respectively. At day 6, the mean plasma HIV-1 RNA change was -1.2 log₁₀ copies/mL in the 50 mg arm; -1.19 log₁₀ copies/mL in the 100 mg arm; and -1.32 log₁₀ copies/mL in the 200 mg arm. In the 200 mg arm, the mean plasma HIV-1 RNA change from baseline to day 10 was 1.77 log₁₀ copies/mL. Small increases in CD4+ cell counts were observed during the treatment period but returned to pre-treatment levels during the 1-month follow-up period. There was no evidence of selection of resistance mutations. All adverse events were mild to moderate and included cold symptoms, headaches, and fatigue; the incidences were similar in the treatment and placebo arms. Long-term toxicity studies were done in rats and dogs; bone marrow toxicity and enteropathy were seen

only in the former. A phase IIb study is currently recruiting patients and will evaluate the efficacy and safety of D-D4FC in treatment-experienced patients in whom previous nRTI-containing regimens had failed.

SN1212/1461. SN1212/1461 is a novel mutagenic deoxyribonucleoside analogue that can inhibit viral growth in tissue culture (Abstract 532). SN1461 is an oral prodrug of SN1212. This agent is not a chain terminator and has an unmodified sugar and thus is unlikely to be affected by lack of affinity of reverse transcriptase for a modified sugar or pyrophosphorolysis, the 2 major mechanisms of nRTI resistance. The EC_{50} of SN1212 is 10 nM to 100 nM. SN1212-treated virus had a more than 50% increase in mutation rate in reverse transcriptase and Env above controls; no SN1212 resistant HIV were isolated. At a dose of 320 μ M, the agent did not result in mitochondrial toxicity. No toxicity was observed in dogs after administration of the prodrug in doses of up to 2g/kg.

SPD754. SPD754 is a deoxycytidine analogue that has shown antiviral activity in treatment-naive patients. At this meeting, new pharmacologic evaluation data in humans, resistance profile, and pre-clinical safety profile of the agent after prolonged administration to monkeys were presented. Bethel and colleagues (Abstract 138) examined the effect of lamivudine on SPD-754 phosphorylation. Twenty-one HIV-1 seronegative individuals received either SPD754 600 mg bid, lamivudine (qd), or both drugs for 4 days, followed by a 7-day washout period. Pharmacokinetic profile of SPD754, and lamivudine and lamivudine-TP, SPD754-triphosphate (TP) were determined in plasma and peripheral blood mononuclear cells (PBMCs). Although the study showed that plasma pharmacokinetics of SPD754 alone and in combination with lamivudine are the same, the intracellular concentration of phosphorylated SPD754 was reduced up to 6-fold in the presence of lamivudine or lamivudine metabolites. Lamivudine decreased the effect of SPD754 against M184V mutant HIV virus by at least 2- to 3-fold. In contrast, SPD754 had no effect on intracellular

lamivudine-TP concentrations. The authors concluded that these findings would preclude the coadministration of lamivudine and SPD754 in the clinical setting. Additional data on the intracellular pharmacokinetics of SPD754-TP in PBMCs of HIV-infected patients were presented by Adams and colleagues (Abstract 599), who showed that the half-life of SPD754-TP in mononuclear cells was approximately 6 to 7 hours, and that intracellular concentrations of SPD754-TP showed some correlation with plasma concentrations. Collins and colleagues (Abstract 526) presented clinical resistance profile of SPD754 after 10 days of monotherapy in antiretroviral naive-patients. Sixty-four patients with CD4+ counts greater than 250 cells/ μ L and plasma HIV-1 RNA levels of 5,000 copies/mL to 1000,000 copies/mL were randomized to 1 of 6 dosage regimen or a placebo. Baseline genotyping was performed on samples from 56 patients, 4 patients had preexisting nRTI-associated resistance mutations (NAMs), 3 of them received the drug, and 1 received placebo. On day 10, the mean drop in plasma HIV-1 RNA levels was 1.18 \log_{10} copies/mL for the 400 mg dose and 1.65 \log_{10} copies/mL for the 1200 mg dose (Cahn et al, 2nd IAS Conference, 2003). On day 10, all 3 patients with the NAM achieved a drop in plasma HIV-1 RNA within 1 standard deviation of the mean change seen in plasma HIV-1 RNA in patients with the wild-type virus at baseline. Overall, no new NAMs emerged after 10 days of monotherapy with SPD754. The authors concluded that this agent warrants further investigation for the treatment of nRTI-resistant virus in combination with other agents. Similarly encouraging were the results of 52 weeks of treatment with SPD754 administered to cynomolgus monkeys, which revealed only minimal and reversible mucocutaneous hyperpigmentation, mild gastrointestinal effects, and minimal changes in red blood cell counts (Abstract 527).

Treatment of Antiretroviral-Naive Patients

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

2NN Substudy

Van Leth and colleagues (Abstract 550) presented additional results from the 2NN trial that compared the antiviral activity of nevirapine, efavirenz, and the combination of nevirapine/efavirenz in the treatment-naive, HIV-infected patients. This substudy analyzed virologic efficacy of each regimen according to baseline CD4+ cell count and plasma HIV-1 RNA level. Patients were randomized to receive a backbone of stavudine/lamivudine with nevirapine qd, nevirapine bid, efavirenz qd, or nevirapine/efavirenz bid. Patients were divided into 3 groups based on their baseline CD4+ counts (<25 cells/ μ L, 25 cells/ μ L to 199 cells/ μ L, or >200 cells/ μ L) and 2 groups according to their baseline plasma HIV-1 RNA levels (<100,000 copies/mL or >100,000 copies/mL). Virologic failure was defined as never reaching plasma HIV-1 RNA levels below 400 copies/mL during follow-up, or rebounding to above 400 copies/mL. At week 48, patients with baseline CD4+ count less than 25 cells/ μ L had a statistically significantly higher risk of virologic failure than did patients with baseline CD4+ counts of at least 200 cells/ μ L ($P=0.04$; hazard ratio [HR]=1.50). Patients with a baseline plasma HIV-1 RNA above 100,000 copies/mL had a statistically higher risk of virologic failure ($P=0.004$, HR=1.48). The nevirapine-only groups were combined because there were no differences in outcome between these arms. In each CD4+ stratum, patients with baseline plasma HIV-1 RNA above 100,000 copies/mL had a higher risk of virologic failure, except in the CD4+ stratum with more than 200 cells/ μ L for nevirapine. There were no statistically significant differences between the nevirapine and efavirenz groups, and the authors suggested that, in patients with advanced disease, there is no convincing evidence that either efavirenz or nevirapine is favorable for first-line treatment.

FORTE Trial

The FORTE trial (Abstract 564), presented by Williams and colleagues, compared the efficacy and safety of the induction/maintenance strategy of 2

Table 2. Selected Results of Trials in Antiretroviral-Naive Subjects

Study (Abstract No.) Description	Regimen/Study Arm (No. 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
FORTE Trial (564) 48-wk study to evaluate the virologic benefit of an induction/maintenance strategy compared with standard 3-drug regimen	I/M therapy (n=62) 2 nRTIs, NNRTI, PI (24-32 wks) \rightarrow drop PI at 24-32 wks ¹ \rightarrow 2 nRTIs, NNRTI	4.92 log ₁₀ (mean)	180 (median)	81% with <50, and 100% with <400 at wk 48	+172 (median)	Mean drop in HIV-1 RNA level significantly greater in the I/M arm. Grade 3 and 4 AEs similar in both arms.
	Standard therapy (n=60) 2 nRTIs, NNRTI	4.96 log ₁₀ (mean)	145 (median)	65% with <50, and 86% <400 at wk 48	+152 ² (median)	
Study 418 (570) 48-wk multicenter, open-label, randomized, comparative trial	Lopinavir 800 mg /ritonavir 200 mg qd (n=115)	4.8 log ₁₀ (median)	214 (median)	70% with plasma level <50	+185 (mean)	The qd group had a higher discontinuation rate (12% vs. 5%). Grade 3 and 4 AEs were similar in both arms, both arms sustained similar increases in total cholesterol and triglyceride levels.
	All patients received tenofovir/emtricitabine	Lopinavir 400 mg/ritonavir 100 mg bid (n=75)	4.6 log ₁₀ (median)	232 (median)	64% <50	
ABCDE Study (716) 48-wk, multicenter, open-label, randomized comparative trial	Lamivudine 150 mg bid or 300 mg qd/efavirenz 600 mg/stavudine 30 mg or 40 mg bid (n=122)	5.21 log ₁₀ (mean)	223 (mean)	64% <50 (ITT analysis) in both arms	+200 (mean increase in both arms)	At wk 48, stavudine arm had higher frequency of lipatrophy than abacavir arm, both combined with lamivudine/efavirenz. More AIDS-defining events in the first 6 mo in abacavir arm.
	Dose of stavudine depended on patient's weight	Lamivudine 150 mg bid or 300 mg qd/efavirenz 600 mg/abacavir 300 mg bid (n=115)	5.21 log ₁₀ (mean)	203 (mean)		
QD triple nRTI (51) 24-wk, pilot study to evaluate potency and safety of an qd regimen of 3 nRTIs Patients were excluded if they had K65R mutation	Didanosine EC 250 mg qd, tenofovir 300 mg qd, lamivudine 300 mg qd (n=24)	4.9 log ₁₀ (median)	133 (median)	0.61 log ₁₀ (overall median reduction at wk 12; n=20)	Not available	20 patients terminated early (median, 16 wks) due to a suboptimal response. Wk-12 resistance testing (n=20) showed 100% with M184I/V, 50% with K65R mutation.

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Table 2. Selected Results of Trials in Antiretroviral-Naive Subjects, continued

Study (Abstract No.) Description	Regimen/Study Arm (No. 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
Tonus Study (52) 12-mo pilot study of qd regimen of triple-nRTI Primary endpoint was wk 48 plasma HIV-1 RNA <50 copies/mL. Study was prematurely interrupted due to high early virologic failure	Abacavir/lamivudine/tenofovir qd (n=40)	4.9 log ₁₀ (median)	221 (median)	33% virologic failure at wk 24 (>400)	Not available	Virologic failure in all patients with baseline HIV RNA-1 >4 log ₁₀ copies/mL. At wk 4, 86% had adequate C _{min} for all 3 drugs. 9/11 developed K65R and M184I/V mutations.
COL40263 (53) Preliminary evaluation of multicenter, open-label study of qd fixed-dose zidovudine/lamivudine/abacavir and tenofovir 22% of subjects discontinued early (8 due to elevated transaminases, 8 due to virologic nonresponse, HIV-1 RNA >400 copies/mL at wk 24)	Interim analysis of 88 subjects at wk 24	5.1 log ₁₀ (median) Required to have plasma HIV RNA >30,000 copies/ml	226 (median)	78% <400 copies/mL (wk 24)* 67% <50 (wk 24) *vs 51% in ESS30009 study	Not available	7/8 subjects with virologic failure had baseline HIV-1 RNA >100,000 copies/mL. 6/8 (75%) had wild-type virus at baseline; at wk 24, 13% had K65R, 25% remained wild-type.

¹ PI was stopped if 2 consecutive plasma HIV-1 RNA levels were below 50 copies/mL.

² The median increase in CD4+ count was not statistically different between arms.

³ Statistically significant difference between the first 2 arms ($P=0.05$).

I indicates induction; M, maintenance; nRTI, nucleoside (or nucleotide) reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; EC, enteric coated; AE, adverse event; qd, once daily; bid, twice daily; ITT, intent-to-treat; wk, week; mo, month.

nRTI with 1 NNRTI and a PI administered for 24 to 34 weeks, followed by 2 nRTIs with one NNRTI, compared with standard therapy of 2 nRTIs and 1 NNRTI in antiretroviral therapy-naive patients. In the induction/maintenance strategy, the PI was stopped if 2 consecutive plasma HIV-1 RNA levels were below 50 copies/mL between weeks 24 and 32. The most common nRTI combinations were didanosine/stavudine (52%) and zidovudine/lamivudine

(42%); the most common NNRTIs were nevirapine (64%) and efavirenz (36%); and the most common PIs were nelfinavir (71%) and lopinavir/ritonavir (27%). This study enrolled and followed up 122 patients with CD4+ counts greater than 25 cells/ μ L for a median of 81 weeks; at baseline the median CD4+ count was 180 cells/ μ L in the induction/maintenance group ($n=62$) and 145 cells/ μ L in the standard therapy group. Mean baseline plasma HIV-1 RNA

levels in the induction/maintenance and standard arms were 4.92 log₁₀ copies/mL and 4.96 log₁₀ copies/mL, respectively. There were 17% and 25% of patients who had baseline plasma HIV-1 RNA levels greater than 300,000 copies/mL in induction/maintenance and standard therapy arms, respectively. Through week 24, 48% of patients in the standard therapy arm experienced virologic failure compared with 31% in the induction/maintenance arm ($P=0.06$); this

difference was even greater at or after 32 weeks (43% vs. 18%, $P=0.002$). At week 48, 65% in the standard therapy arm and 81% in the induction/maintenance arm had plasma HIV-1 RNA levels less than 50 copies/mL ($P=0.07$); the mean fall in plasma HIV-1 RNA level was 0.86 \log_{10} copies/mL greater in the induction/maintenance arm ($P=0.01$). The median increase in CD4+ count at 48 weeks was 172 cells/ μ L and 152 cells/ μ L in the induction/maintenance arm and the standard arm, respectively; the differences between the 2 treatment arms were not statistically significant. Only 5 patients were lost to follow up; by week 48, 83% of patients in the induction/maintenance arm and 84% of patients in the standard arm adhered to the assigned treatment. In the induction/maintenance group, 58% of patients stopped their PIs as planned at a median time of 26 weeks. The incidences of grade 3 or 4 adverse events were similar in both study arms, and they included elevation of liver enzymes, vomiting, and peripheral neuropathy. There was also no difference in the number of patients progressing to a new AIDS event or death. To interpret the efficacy of the induction/maintenance arm properly, a control arm using the maintenance regimen throughout the study would be needed.

Study 418

Gathe and colleagues (Poster 570) presented the week-48 results from the 418 study. This was a multicenter, open-label, randomized trial that compared the antiviral activity and safety of qd lopinavir/ritonavir and bid lopinavir/ritonavir in antiretroviral-naive, HIV-1 infected patients. A total of 190 patients with a screening plasma HIV-1 RNA level greater than 1,000 copies/mL and no CD4+ cell count criteria were randomized to receive lopinavir 800 mg/ritonavir 200 mg qd ($n=115$), or lopinavir 400 mg/ritonavir 100 mg bid ($n=75$). All patients received a backbone of qd tenofovir and emtricitabine. The overall median baseline plasma HIV-1 RNA level was 65,000 copies/mL, approximately 45% of patients had baseline CD4+ counts below 200 cells/ μ L, and 38% had a baseline plasma HIV-1 RNA level above 100,000

copies/mL. Over 20% of patients were women and about 55% were white.

At week 48, according to an intent-to-treat (ITT) analysis, the proportions of patients who achieved a plasma HIV RNA level below 50 copies/mL were 70% in the qd arm and 64% in the bid arm; the 95% confidence interval (CI) for the difference in responses between the 2 arms (-7%, 20%) met the protocol definition for non-inferiority of the qd regimen. No statistically significant differences were observed among the study arms with respect to the CD4+ cell count changes from baseline. Genotypic testing was performed for patients with plasma HIV-1 RNA levels above 500 copies/mL occurring at any time during week 12 to 24 (5 in each arm). There was no lopinavir or tenofovir resistance, and emtricitabine resistance emerged in 1 patient in each group.

Over 48 weeks, the proportion of subjects who discontinued the study was 19% in the qd and 25% in the bid arm; a higher rate of discontinuation due to adverse events was seen in the qd group (12% vs. 5% in the bid group), and higher rates of nonadherence and loss to follow-up were observed in the bid group (12% vs. 4% in the qd group). The most common adverse clinical event was diarrhea, which occurred in 16% in the qd arm and 5% in the bid arm ($P=0.04$). There were no statistically significant differences in the proportions of grade 3 or 4 adverse clinical events between the 2 arms; laboratory hepatobiliary toxicity (elevation of transaminase levels) occurred in 5% and 3% of patients on the qd and bid regimens, respectively. Both arms sustained significant mean lipid elevations from baseline (in total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels), but this did not result in significant changes in the 10-year coronary heart disease risk based on the Framingham Heart Study calculations.

Treatment of Antiretroviral-Experienced Patients

Lopinavir Inhibitory Quotient

Bertz and colleagues presented data correlating the lopinavir inhibitory quo-

tient (C_{trough} /protein adjusted IC_{50} ; IQ) and virologic response to two lopinavir/ritonavir-based salvage regimens (633 mg/166 mg or 400 mg/300 mg each with + 2 or 3 nRTIs) (Abstract 134). The patients were nRTI-, NNRTI- and PI-experienced and had a median fold-change in lopinavir IC_{50} of 4.1 (range 0.7-238). Overall, 58% of participants achieved a plasma HIV-1 RNA below 400 copies/mL, and this was similar in each arm. Details of the virologic outcomes will be presented at a future conference. In multivariate analyses, higher lopinavir IQs and more active nRTIs were associated with an improved virologic response. In contrast, lopinavir pharmacokinetic parameters were not associated with virologic response. This study provides evidence that higher doses of PIs may overcome resistance in some instances.

Lopinavir/Ritonavir vs. Atazanavir/Ritonavir

DeJesus and colleagues presented the 48-week results of a comparison of atazanavir plus ritonavir or saquinavir, with lopinavir/ritonavir in antiretroviral-experienced patients (Abstract 547). Subjects must have had more than 2 previous antiretroviral therapy regimens that included nRTI, NNRTI, and PI failure. They must have had a CD4+ count of more than 50 cells/ μ L and a plasma HIV-1 RNA above 1,000 copies/mL. Subjects replaced their failing PI with atazanavir/ritonavir (300 mg/100 mg qd), lopinavir/ritonavir (400 mg/100 mg twice daily) or atazanavir/saquinavir (400 mg/1000 mg qd) for 2 weeks. The nRTIs were then changed to tenofovir and an additional nRTI. The HIV-1 RNA at baseline was approximately 4.4 \log_{10} in all 3 arms, and the median CD4+ counts were 317 cells/ μ L, 283 cells/ μ L, and 286 cells/ μ L, respectively.

Similar to the 24-week results presented previously, the atazanavir/saquinavir arm was found to be inferior to the other 2 arms at 48 weeks. The time-averaged change in plasma HIV-1 RNA from baseline was not different between the atazanavir/ritonavir and lopinavir/ritonavir arms. The change from baseline plasma HIV-1 RNA at week 48 was -1.93 \log_{10} for the atazanavir/ritonavir arm and -1.87 \log_{10}

for the lopinavir/ritonavir arm. In 56% and 38% of the atazanavir/ritonavir arm HIV-1 RNA levels were below 400 copies/mL and below 50 copies/mL at 48 weeks, respectively, compared with 58% and 46% of lopinavir/ritonavir-treated subjects. The mean change in CD4+ count was 110 cells/ μ L and 121 cells/ μ L in the atazanavir/ritonavir and lopinavir/ritonavir groups, respectively. Forty-nine percent in the atazanavir/ritonavir arm experienced elevations in bilirubin more than 2.5 times the upper limit of normal, compared with less than 1% in the lopinavir/ritonavir arm. The atazanavir/ritonavir arm was less likely to use lipid-lowering agents (8% vs. 19% in the lopinavir/ritonavir arm), and patients in this group had lower triglyceride levels (a 4% reduction from baseline compared with a 30% increase for the lopinavir/ritonavir arm) and total cholesterol (an 8% reduction from baseline compared with a 6% increase).

Lamivudine in Salvage Regimens

In vitro data suggest that the most common lamivudine-associated mutation, M184V, confers increased susceptibility to certain nRTIs, namely tenofovir, zidovudine, and stavudine, but not abacavir or didanosine. Consequently, lamivudine is often used in salvage regimens despite documented genotypic resistance. Dragsted and colleagues presented data on the use of lamivudine in salvage therapy for patients in whom a lamivudine-containing regimen was failing (Abstract 549). There were 133 subjects in whom a lamivudine-containing regimen failed. They were randomized to continue or to discontinue lamivudine in addition to starting a new antiretroviral regimen that was chosen prior to randomization.

At baseline, the median plasma HIV-1 RNA was 4.0 log₁₀ copies/mL and the median CD4+ count was 310 cells/ μ L. At 48 weeks, 54 (83%) in the lamivudine arm and 60 (91%) of the no-lamivudine arm were still on their randomized treatment. There was no significant difference in the time-averaged change in HIV-1 RNA from baseline (-1.4 vs. -1.5 log₁₀), percent below 400 copies/mL (66% vs. 65%), percent below 50 copies/mL (52% vs. 44%), or protocol-defined virologic failure. The

M184V was maintained in more than 80% of subjects continuing to take lamivudine, but it was lost in the majority of the no-lamivudine arm (eg, present in <10% of follow-up samples tested).

Predictors of Response to Antiretroviral Therapy

Moore and colleagues presented data from the Johns Hopkins Cohort on the response to an initial antiretroviral regimen in HIV-infected persons over the age of 50 years (Abstract 556). Individuals over the age of 50 years were more likely to reach a plasma HIV RNA below detection than those aged 35 to 50 or younger than 35 (62.5% vs. 53.8% and 48.9%, $P = .01$), and were more likely to have durable virologic suppression (40.2% vs. 29.7% and 27.5%, $P = .01$). The CD4+ cell count changes were similar among the 3 groups. Mortality was significantly higher among those aged 50 or older, but this was primarily due to non-HIV related illness. Older age was also associated with an improved virologic response in AIDS Clinical Trials Group (ACTG) 388 (zidovudine/lamivudine plus: indinavir, efavirenz/indinavir, or nelfinavir/indinavir; Abstract 553).

Kaufmann and colleagues looked at CD4+ cell count rise in 326 participants in the Swiss HIV Cohort Study who maintained HIV-1 RNA levels above 1000 copies/mL for 5 years after an initial antiretroviral therapy regimen (Abstract 557). They found that 37.5% did not reach a CD4+ count of above 500 cells/ μ L at 5 years. This was associated with older age (odds ratio [OR] 3.0/10 years older) and with a lower baseline CD4+ cell count (OR, 0.54 per 100 CD4+ cells higher at baseline).

Triple-Drug Class Failure

Mocroft and colleagues (Abstract 554) presented data on behalf of the EuroSIDA study group. They examined the time to triple-drug class failure from enrollment in the cohort. At 6 years of follow-up, 24.1% of patients who were antiretroviral-experienced (ie, with monotherapy or dual therapy) prior to

starting potent antiretroviral therapy had triple-drug class failure compared with 11.9% who were treatment-naive. Among patients who were treatment-experienced at baseline, prolonged exposure to nRTIs increased the risk of triple-drug class failure. Among patients who were treatment-naive, lower baseline CD4+ cell count and higher plasma HIV RNA level were associated with an increased risk of developing triple-drug class failure.

Changes in the Initial Antiretroviral Regimen

Moore and colleagues (Abstract 558) described the choices for initial antiretroviral regimens and the overall likelihood of virologic suppression from 1996 to 2002 among patients in the Johns Hopkins database. The use of a single, unboosted PI declined dramatically, from 85% in 1996 to 13% in 2001/2002. Concomitantly, there were increases in the use of NNRTI-based regimens (0% to 59%) and triple-nRTI regimens (0% to 16%). Ritonavir-boosted PI regimens accounted for 7% of initial antiretroviral regimens in 1996, 18% in 1997 and 1998, 15% in 1999 and 2000, and 13% in 2001-2002. These changes were associated with improved virologic outcomes. Forty-five percent of patients starting antiretroviral therapy in 1996 achieved a plasma HIV RNA below detection by 6 months compared with 73% who started in 2001 or 2002. Factors associated with achieving an HIV RNA level below detection by 6 months also included use of NNRTI or a boosted PI and no prior use of nRTIs.

Primary HIV Infection

Pilcher and colleagues (Abstract 20) reported experience with the Screening and Tracing Active Transmission (STAT) program conducted in North Carolina. In this study, North Carolina's 110 public HIV-testing sites added nucleic acid testing (NAT) to allow early detection of HIV-1 infection. Following enzyme immunoassay (EIA)/Western blot testing, antibody specimens were pooled 1:10:90 in pools of 100 specimens/pool and screened by NucliSens NAT. Acute infection was defined as HIV-1 RNA-positive and HIV-1 antibody negative. A

prevalence of HIV-1 infection of 56.7 per 10,000 was detected; of these, 21 individuals were diagnosed with acute infection. The NAT offered an additional 4.1% to the diagnostic yield of antibody testing (11.1% in jails, 6.4% in sexually transmitted diseases [STD] clinics). High transmission areas and factors associated with primary HIV infection in North Carolina were identified: rural and major urban areas and major tracking routes, male sex, men who have sex with men (MSM) status, multiple partners, African-American ethnicity, and age younger than 24 years. The STAT program offered a new model for increasing effectiveness of the volunteer counseling- and testing-based HIV surveillance and for targeting prevention programs.

Little and colleagues (Abstract 384) attempted to elucidate biologic factors that influence the relative transmissibility of certain viral variants from an HIV-1 infected source to a new index patient. It had been proposed (Derdeyn et al, 10th CROI, 2003) that the transmitted virus has more variable loop deletions in the *env* gene, and less neutralization resistance than most viruses from the donor individual. This study evaluated 10 patients with primary infection, within 50 days of seroconversion, and their 8 phylogenetically linked sexual partners. The *env* sequence of the source and the recipient patient clustered together and the genetic divergence between them was less than 2%. There were no significant differences between envelope lengths or the number of glycosylation sites between the source and the recipient partner. Neutralizing antibody responses were weak in both the source and the recipient, and wherever differences were seen, the recipient virus was more neutralization-sensitive. The authors concluded that neutralizing antibodies probably do not represent a critical factor in the selection of transmitted virus.

Ritola and colleagues (Abstract 386) investigated the viral complexity present during primary HIV-1 infection. The V1/V2 and V3 variable regions of *env* were determined for 109 subjects with primary infection, acquired mostly through homosexual contact (n = 95). Fifty-three percent of patients infected following homosexual contact had mul-

tiples V1/V2 variants; 57% of women versus 15% of men infected through heterosexual contact had multiple variants. Eighty-two percent of all subjects had a single V3 variant. Three percent of subjects had CXCR4-using variants. Based on these results the authors concluded that the type of mucosal surface exposed to the virus in combination with the sex of the transmitter influences the number of variants transmitted.

Lichterfield and colleagues (Abstract 393) evaluated the predominant target of early CD8+ T-cell response in 10 individuals with acute, early, and chronic infection. Ninety-four percent and 46% of the HIV-1 specific CD8+ T-cell responses were directed against Nef in patients with acute and chronic infection, respectively, and patients with untreated chronic infection had broadly diversified CD8+ T-cell responses. The patients with acute infection, who initiated therapy, maintained the immunodominance of Nef, and those who remained treatment naive after 1 year exhibited a steady increase in magnitude and breadth of HIV-specific CD8+ T cell responses. The authors postulate that this specificity of CD8+ T-cell responses during acute infection may become important in vaccine design.

Primary Infection: Response to Treatment

Voirin and colleagues (Abstract 23) presented data from 3 prospective primary HIV-1 infection cohorts in Lyon, Montreal, and Sydney. Patients (n = 203) were divided into 3 groups: starting antiretroviral therapy when symptomatic with acute retroviral syndrome (n = 51); starting therapy after the resolution of symptoms (n = 117); and receiving no therapy (n = 35). The mean overall baseline plasma HIV-1 RNA level was 4.49 log₁₀ copies/mL, and they were not statistically different across study groups. After 3 years of follow-up, there were no differences in plasma HIV-1 RNA level and CD4+ cell count between patients who initiated antiretroviral therapy. The decay in plasma HIV-1 RNA level from baseline to 6 months was more dramatic in the group treated during acute retroviral syndrome, but this was not statistically sig-

nificant (P = 0.4). The group that did not receive antiretroviral therapy had higher plasma HIV-1 RNA levels, and lower CD4+ cell counts at the end of a 3-year period.

Desquilbet and colleagues presented data on behalf of the French PRIMO study group (Abstract 397). They compared patients who initiated antiretroviral therapy within 6 months of seroconversion with patients from the SEROCO cohort who did not receive antiretroviral therapy after seroconversion. After adjusting for baseline plasma HIV-1 RNA level, patients who received 24 months of antiretroviral therapy followed by a 12-month treatment interruption had an estimated plasma HIV-1 RNA level of 3.75 log₁₀ copies/mL compared with 3.94 log₁₀ copies/mL in the untreated cohort. An adjusted analysis did not show a clear benefit of early treatment, and authors suggested that a randomized trial is needed to evaluate the long-term benefits of interruption of early antiretroviral therapy.

Primary Infection: Treatment Interruptions

Kaufman and colleagues (Abstract 24) presented follow-up data from the observational study of supervised treatment interruptions (STIs) during acute HIV-1 infection. Fourteen patients who were treated during acute infection and subsequently had a treatment interruption after virologic control followed by retreatment when plasma HIV-1 RNA remained above 5000 copies/mL for longer than 3 weeks (or was higher than 50,000 copies/mL at any point) have now been followed up for a median of 3.5 years. When treatment was stopped, only 11 patients maintained control of plasma HIV-1 RNA levels for at least 90 days. Only 3 patients achieved a maximal treatment interruption of 720 days, and they did not differ from the remaining patients by human leukocyte antigen (HLA) allele or baseline plasma HIV-1 RNA levels. The rate of CD4+ cell decline was variable. The rise in CD8+ T-cell response during the first, second, and third supervised treatment interruptions did not predict time to failure.

Hoen and colleagues (Abstract 395) presented the final results of the PRIM-

STOP pilot trial from France. Twenty-nine patients received a regimen of didanosine/stavudine/nelfinavir/hydroxyurea for 34 weeks, followed by 3 consecutive periods of 2, 4, and 8 weeks off antiretroviral therapy, separated by 12 weeks on treatment, discontinuation of antiretroviral therapy at week 84, and follow-up to week 108. Three patients were lost to follow-up. Of the 26 who completed the study all remained off therapy at week 108, and 1 had a plasma HIV-1 RNA level of less than 50 copies/mL. Only female sex was associated with a plasma HIV-1 RNA level of less than 1000 copies/mL at week 108. Three patients developed a major PI resistance (L90M) and hydroxyurea had to be stopped in more than half of the patients due to severe neuropathy.

Grey and colleagues (Abstract 399) evaluated virologic and immunologic predictors of initial response during structured treatment interruption in patients with acute and early HIV-1 infection. Lower baseline viral load and a shorter time to plasma HIV-1 RNA suppression on therapy were predictive of a longer time to rebound. Maintenance of a central pool of CD28+ CD8+ T-cells, rather than expansion of CD28+ effector T-cells, was associated with better control on therapy.

Superinfection

Several studies addressed superinfection during early HIV-1 infection. Smith and colleagues documented the frequency of HIV superinfection within clade B in a cohort of 56 antiretroviral-naïve subjects deferring antiviral therapy (Abstract 21). The majority of the individuals were MSM. Superinfection was presumed where baseline and follow-up samples appeared phylogenetically distinct (mean 313, range 177–597 days apart). Evidence for superinfection was strengthened by clonal analysis and dye-primer length polymorphism analysis of HIV *env* and population-based *pol* sequencing. Superinfection was identified in 3 of 56 (6.5%) at 5 to 15 months after infection. In 2 cases, a wild-type strain was replaced by a resistant one. In the other, a drug-resistant virus replaced the wild-type strain. All individuals were men

whose risk factor was homosexual exposure. Plasma HIV-1 RNA levels increased (mean 1.6 log increase) and CD4+ counts decreased (mean 132 cell/μL decrease) within 6 months of acquiring the superinfecting strain. It was estimated that superinfection occurred 5 to 13 months after the estimated date of primary infection.

Daar and colleagues (Abstract 394) described 1 patient initially infected with a multidrug-resistant subtype B HIV-1 virus who within 6 months of seroconversion had superinfection with a phylogenetically distinct, wild-type strain and had a modest increase in plasma HIV-1 RNA. Analyses of immune responses and sequence changes at several cytotoxic T lymphocyte (CTL) epitopes demonstrated that the new strain had a higher viral set-point but similar replicative capacity than the original strain; epitopes in Gag and Nef had key sequence differences in the superinfecting strain compared with the original HIV-1 virus. Distinct CTL responses were observed between the 2 strains, suggesting that this was more likely a distinguishing characteristic between the 2 strains than differences in replication capacities.

Similar data were presented by Gottlieb and colleagues (Abstract 454) using data from the Multicenter AIDS Cohort Study (MACS). Samples from 32 seroconverters were retrospectively evaluated using a combination of heteroduplex mobility assay, sequencing of the envelope C2-V5 region, and phylogenetic methods. Presumed superinfection with a second HIV-1 subtype B strain was detected in 1 individual (3%) at 1.3 years post infection. The newer isolate was also identified as an X4/syncitium-inducing phenotype. Within 6 months, there was rapid replacement of the initial virus by the superinfecting virus that was not explained by random genetic drift.

In 58 subjects from the Women's Interagency HIV study (WIHS) who reported or did not report injection-drug use (IDU), genetic differences in circulating HIV were evaluated (Abstract 952). Clonal analysis (≥ 5 clones/sample) of the HIV protease was made from samples with at least 10,000 HIV-1 RNA copies/mL. Among subjects with CD4+ counts above 650 cells/μL, genetic

diversity was statistically significantly greater ($P < 0.03$) in IDU than among non-IDU subjects. This difference was not observed at lower CD4+ levels.

Pao and colleagues (Abstract 392) described the epidemiology of 104 individuals in the United Kingdom diagnosed with primary HIV infection (PHI) between 1999 and 2003. The median age was 36 years and 96% were men with 87.5% identifying MSM as their risk behavior and 6% identifying IDU as theirs. In the 3 months prior to PHI, 42% had 3 or more sexual contacts and 34% had more than 1 STD. Thirteen of 104 (12.5%) had antiretroviral resistance. Phylogenetic analyses of *pol* identified 16 clusters from 35 individuals (97.5% MSM). Compared with non-clustered isolates, clustered isolates were more frequently derived from significantly younger individuals, and those who reported greater numbers of sexual contacts and higher rates of unprotected anal intercourse. The authors suggest these data may assist planning of future preventative strategies.

Sagar highlighted the stable coexistence of distinct HIV strains among untreated heterosexually-infected sex workers in Mombasa, Kenya (Abstract 385). Ten subjects were evaluated, 5 with relatively diverse envelope sequences and 5 with more homogeneous sequences. Among the latter, significantly greater diversity was also observed among *gag* and *pol* sequences (average pairwise distances, $P < .001$). Further, these relationships were preserved over the ensuing 3 years in the 6 subjects with available data.

Treatment Strategies

Drug Reduction Strategies

Launay (Abstract 649) evaluated whether a strategy of reduced drug pressure could stabilize evolution of resistance in a multicenter pilot study in France. In the 26 subjects, a regimen including PI was failing, they had plasma HIV-1 RNA above 10,000 copies/mL, and they had drug resistance genotypes predicting activity of no more than 1 drug. All subjects were switched to

lamivudine with low dose indinavir/ritonavir 200 mg/100 mg bid. Pharmacokinetic management involved week-2 sampling of indinavir trough with adjustment to achieve a trough in the range 150 ng/mL to 350 ng/mL (using the estimated inhibitory quotient [IQ] of 0.5 for a multi-PI resistant virus). The median CD4+ cell change to 24 weeks was $-49/\mu\text{L}$ ($P < 0.001$). However, the mean CD4+ count slope was equivalent to the slope in the 6 months prior to entry ($P < 0.001$). The median increase in plasma HIV-1 RNA was 0.22 log₁₀ copies/mL. However, 16 of 25 subjects had trough indinavir values below 150 ng/mL, requiring corrective dose adjustment of indinavir to 400 mg bid. Resistance profiles were reported as being stable for the duration of the study. A cost-reduction of antiretroviral therapy of 76% per day was reported.

Late Intensification: The Addition of Abacavir to a Stable Treatment Regimen With Sustained Plasma HIV-1 RNA Level Suppression.

Hammer and colleagues (Abstract 56) presented the results of ACTG 372A. This was a randomized, double-blind, placebo-controlled trial enrolling 229 individuals with plasma HIV-1 RNA levels of less than 500 copies/mL and who were on stable therapy with zidovudine (or stavudine)/lamivudine/indinavir at 800 mg 3 times a day (tid). Subjects were randomized to continue their therapy and add abacavir or abacavir placebo. The composite endpoint was the time to virologic failure (confirmed HIV-1 RNA > 200 copies/mL) or permanent treatment discontinuation. The median baseline CD4+ counts in the abacavir and abacavir placebo arms were 245 cells/ μL and 252 cells/ μL , respectively. A total of 180 subjects (79%) completed the study, with 124 (54%) completing the study on their assigned treatment. The median follow-up time was 227 weeks. Comparing the 2 study arms, there were no differences in CD4+ count increases during follow-up. Nor were there differences in the proportions with virologic failure (ITT log rank $P = 0.2$), with HIV-1 RNA levels below 50 copies/mL or with HIV-1 RNA levels below 6 copies/mL at 48 weeks

($n = 98$). Nor were there differences in the levels of proviral HIV-1 DNA in subjects in each arm with sustained suppression of HIV-1 RNA levels below 50 copies/mL ($n = 82$). Among 69 subjects experiencing virologic failure, resistance testing demonstrated no significant differences in the relative frequencies of K65R (0%, 0%), L74V (0%, 7%), and M184V (41%, 41%) in the abacavir and placebo arms, respectively. Nephrolithiasis (overall, 17%) was observed in 14 abacavir recipients and 26 abacavir placebo recipients ($P = 0.037$). The authors suggest that these results do not support a strategy of late intensification with abacavir in those with stable plasma HIV-1 RNA level suppression.

Structured Treatment Interruptions in Antiretroviral-Experienced Persons

The Community Programs for Clinical Research on AIDS (CPCRA) 064 study previously demonstrated the lack of benefit of 4-month structured treatment interruption (STI) prior to starting a new regimen in subjects with moderately advanced disease. Lawrence and colleagues (Abstract 665) described the changes in genotypic resistance patterns in those with STIs that were 4 months or more ($n = 93$), 2 to 4 months ($n = 28$), and for less than 2 months ($n = 8$). STI termination prior to 4 months was recommended if the CD4+ count fell more than 50% from baseline to follow-up. In the group with STI of 4 months or more, the mean HIV-1 RNA levels were significantly lower than the means of the other 2 groups (4.9 log copies/mL versus 5.3 log₁₀ copies/mL, respectively; $P = 0.003$). Longer STI (≥ 4 months) was associated with greater reduction in the number of major mutations (from 9.9 to 4.5) and an increase in the mean number of drugs to which the virus was susceptible (from 2.0 to 9.8).

Benson and colleagues (Abstract 58) presented the week-48 results of ACTG 5806. In this study, 41 patients with a plasma HIV-1 RNA level greater than 5,000 copies/mL, and a history of virologic failure on at least 2 prior antiretroviral regimens, were randomized into 1 of 2 treatment arms: 16-week struc-

tured treatment interruption (STI) followed by an optimized antiretroviral regimen that was selected based on results of resistance testing ($n = 21$); or immediate initiation of optimized antiretroviral therapy ($n = 20$). The primary endpoint was plasma HIV-1 RNA level below 400 copies/mL at week 48. The median baseline CD4+ count and plasma HIV-1 RNA level were 225 cells/ μL and 38,000 copies/mL, respectively. At week 48, the proportion of subjects with plasma HIV-1 RNA levels below 400 copies/mL was 19% and 33% in the STI and no-STI arms, respectively ($P = 0.44$); the median drop in plasma HIV-1 RNA level was 0.65 log₁₀ in the STI arm, and 1.15 log₁₀ in the no-STI arm ($P =$ not significant). The median increase in CD4+ count from baseline was 10 cells/ μL and 17.5 cells/ μL in the STI and no-STI arms, respectively. In the STI arm, 18 subjects had genotypes performed at end of the treatment interruption; reversion of baseline mutations was seen in 5 of 18, partial reversion in 7 of 18, and little or no reversion in 6 of 18 patients. At week 48, in patients with virologic failure despite reversion of baseline mutations, phylogenetic analyses demonstrated that resistance mutations clustered with the virus population at entry and not at the end of the STI. Three-drug class resistance occurred in patients with virologic failure despite antiretroviral therapy with only 2 drug classes, indicating linkage of mutations on the same genome. The authors concluded that the persistence of resistant virus despite STI is a likely explanation for the ineffectiveness of STI in patients with multi-drug-resistant HIV-1.

Calvez and colleagues (Abstract 661) compared the rates of reversion to wild-type virus at 26 weeks, by antiretroviral class, in 19 subjects with stable on-treatment viremia. The median CD4+ count was 61.5 cells/ μL and the median HIV-1 RNA was 5.1 log₁₀ copies/mL. The median number of nRTI, NNRTI, and PI mutations was 7, 2 and 4, respectively. The shift to wild-type virus was faster for PIs, intermediate for NNRTIs, and slowest for nRTIs ($P < 0.05$). Reversion at reverse transcriptase codons 41, 215, and 219 was uncommon. These data underline the complex dynamics of the circulating, resistant HIV quasispecies.

The Emergence of Drug Resistance With Repeated STIs

With the exception of the Agence Nationale de Recherches sur le Sida (ANRS) 097 study in experienced persons with virologic failure, trials have largely failed to demonstrate clinical benefit to STIs. However, interruptions in therapy do occur in clinical practice for a variety of reasons. The potential risks of treatment interruptions in relation to the emergence of resistance were highlighted by several presentations at the conference.

The ISS PART study (Abstract 552) is an ongoing, randomized, multicenter clinical trial comparing continuous versus intermittent therapy in 273 subjects with plasma HIV-1 RNA below 400 copies/mL who are on a stable first regimen. An STI strategy was undertaken by 136 subjects comprising 4 STIs of 1, 1, 2, and 2 months each separated by 3 months of treatment. Of these subjects 39 (29%) had resistance mutations at STI. Among nRTI-treated subjects, 136 (3%) had the T215Y mutant; of those taking lamivudine, 123 (16.3%) developed M184V; of PI-treated subjects, 59 (10.2%) developed L90M or M46I; and of those on NNRTIs, 101 (7%) developed K103N. Of the 39 subjects with mutations, 11 (28%) were detected in lymphocyte DNA prior to the STI. The proportion with virologic failure (HIV-1 RNA >400 copies/mL at the end of treatment phase) was greater for those demonstrating mutations than for those without mutations, 13 of 39 (33%) and 12 of 97 (12.4%), respectively ($P=0.004$).

Antiretroviral Drug Resistance and Replication Capacity

Transmission of Drug-Resistant Virus and Prevalence Studies of Drug Resistance in Treatment-Naive Patients

For the Options project, Kozal (Abstract 35LB) described transmission-risk behavior patterns in HIV-infected individuals and their relationship to the potential transmission of drug-resistant virus. Of the 333 subjects enrolled between 2000 and 2003 98 (27%) had resistance to at least 1 class of drug. Of those followed up for more than 6

months, 19 had drug resistance and engaged in sex with an HIV-seronegative or serostatus-unknown partner. These individuals engaged in 423 such events representing 38% (423 of 1,116) of all high-risk sex events. These data suggest that a likely source of transmitted resistance is a small core group of individuals in clinical care with known resistance and ongoing HIV transmission behaviors.

Little and colleagues (Abstract 36LB) described the persistence of transmitted drug-resistant virus among subjects with PHI who deferred antiretroviral therapy. Twelve subjects were identified. The median follow-up, plasma HIV-1 RNA, CD4+ count, and replication capacity were 310 days, 5.2 log₁₀ copies/mL, 542 cells/μL and, 84%, respectively. NNRTI, PI, and nRTI resistance was observed in 10, 4, and 5 subjects, respectively. Reversion of resistance was seen infrequently; 1 of 10 isolates with K103N reverted to N103K/N and 3 of 5 nRTI mutants also reverted (T215F⇒T215Y/C [n=1], M184V⇒V184M or M/V [n=2 of 5]). Further, the time for these events to occur was long, a median of 362 days. No reversion of PI resistance mutations was observed.

For the Duke-University of North Carolina-Emory PHI Consortium, Hicks and colleagues (Abstract 682) described the primary phenotypic resistance profiles in 30 residents of North Carolina with PHI diagnosed between January 1998 and January 2003. Resistance comparisons for seroconversions up to June 2000 (n=12) and from July 2000 (n=18) were made. Resistance was more common with seroconversions up to June 2000 ($P=.018$) and for whites compared with all others ($P=.018$). Overall 4 of 30 subjects had resistance to at least 1 drug; all 4 were diagnosed prior to July 2000. The authors noted an apparent reduction in the rate of transmitted resistance more recently.

A complementary presentation by de Mendoza noted an overall reduction in the rate of transmitted resistance in 128 consecutive newly HIV-infected individuals seen between January 1997 and December 2003 in 4 clinics in Spain (Abstract 681). Of these subjects, 19 (15%) had genotypic evidence of resistance; and 18, 2, and 4 had nRTI,

NNRTI, or PI resistance mutations, respectively. The rates of drug resistance in 1997 and 2003 were 33.3% and 10%, respectively. The authors noted a negative correlation with yearly rates of those with plasma HIV-1 RNA below 50 copies/mL and those of primary drug resistance, ($r=-0.87$, $P=0.054$).

Yerly and colleagues (Abstract 680) struck a cautionary note concerning interpretation of changes in the prevalence of transmitted drug resistance. This group evaluated factors modulating the prevalence of resistance within the Swiss HIV cohort. There were 505 subjects with HIV seroconversion diagnosed between January 1996 and December 2003 who were evaluated. Overall, nRTI mutations were most frequently seen, and among these, the most common change was at codon 215 (17 of 31; 55%). Factors impacting prevalence included clustering of resistance (5 subjects in Lausanne in 2001 with mutations M41L/T215D); the influx into this population of non-B subtypes with lower levels of resistance; and the absolute HIV-1 RNA level, with greater proportions of subjects in care with plasma HIV-1 RNA below 400 copies/mL.

Cane reported long-term resistance profiles in 16 patients with resistance mutations noted at primary infection (Abstract 684). Three of these 16 patients were infected with virus resistant to 3 classes, and 2 of the 16 had NNRTI resistance only. Mutations at reverse transcriptase codons 41, 69, 215, and 219 were seen in 5, 4, 6, and 3 subjects, respectively. The median time to follow-up sequencing was 20.5 months (range, 2-120), at which time resistance patterns remained stable on no therapy. Loss of only 3 mutations was observed at follow-up: V62A, C181Y, and Q219K. One further isolate demonstrated a switch from T215Y⇒C concurrent with a 10-fold increase in HIV-1 RNA.

For the CASCADE cohort (Concerted Action on Seroconversion to AIDS and Death in Europe), Masquelier described the complete resistance profiles of 432 subjects for whom resistance testing was performed within 18 months of seroconversion (occurring between 1987 and 2003; Abstracts 683, 684). Transmitted resistance was observed in 54 of 432 (12.5%) individuals. In 47 of

54 (87%) resistance was to one class (34 to nRTI, 8 to NNRTI, and 5 to PI); in 1 of 54 (2%) there was resistance to 3 classes. Non-subtype B strains were more recently observed (after August 1999) and more likely to occur as a result of heterosexual transmission. A trend toward more frequent resistance over time was noted ($P = .07$).

Bezemer and colleagues (Abstract 679) described limited recent prevalence of drug resistance in 100 newly diagnosed HIV infections in Amsterdam from 1994 to 2002. From 1996 onward, only revertants at reverse transcriptase codon 215 were seen. Prior to 1998, 20% of new infections had genotypic evidence of resistance compared with 6% from 1998 onward. The median plasma HIV-1 RNA was 4.4 log₁₀ copies/mL compared with 5.0 log₁₀ copies/mL in drug resistant versus non-resistant isolates, respectively ($P = .036$).

The Impact of Transmitted Resistance on First Regimen Outcome

Borota-Esoda (Abstract 672) examined the impact of baseline resistance in an international study of antiretroviral-naïve subjects receiving efavirenz/didanosine with either emtricitabine ($n = 285$) or stavudine ($n = 285$). The authors evaluated baseline resistance in those subjects followed up to week 60 and who were unable to achieve, or rebounded from, plasma HIV-1 RNA levels below 400 c/mL. Among these 546 subjects, baseline genotypic resistance to nRTI, NNRTI, or both classes was seen in 6.2%, 8.8%, and 1.5%, respectively. For both study arms, the proportions with virologic failure were significantly greater in these arms with resistance to NNRTIs, nRTIs, or both classes. For those in the emtricitabine/didanosine/efavirenz arm, the proportion with treatment failure with K103N at entry was 43% and without K103N was 4% ($P = 0.001$). For those in the stavudine/didanosine/efavirenz arm, the proportion with treatment failure with the K103N at entry was 71% and without K103N was 12% ($P = 0.001$).

The impact of transmitted resistance on therapy outcome was also evaluated by Pillay within the CASCADE study reported above (Abstract 685). Among

the 199 persons starting therapy between June 1996 and June 2003, 26 (13.7%) had genotypic evidence of resistance. The most common mutations were at codons 41 ($n = 9$) and 215 ($n = 15$). The median time from infection to initiation of potent antiretroviral therapy was 244 days. At a median time on therapy of 94 days, 90% of subjects had plasma HIV-1 RNA below 500 copies/mL. No difference in virologic success was observed between those with and without resistance. The authors speculate that primary resistance may complicate later treatment failure.

Resistance Following Sequential Regimens

The rates of resistance at baseline and at follow-up on sequential antiviral regimens were explored by Johnson and colleagues in ACTG 384 (Abstract 662). This study evaluated a variety of linked first and second regimens in antiretroviral-naïve subjects. The first nRTI combinations were either stavudine/didanosine or zidovudine/lamivudine. The nRTIs were coadministered with efavirenz, nelfinavir, or both. Resistance mutations were observed at baseline in only 1% (10 of 899) subjects. Among those receiving 3 drugs, 44% to 72% of subjects had virologic failure with wild-type HIV sequences. Thymidine analogue-associated mutations (TAMs) were seen at failure in approximately 3% of all regimens, and K65R and L74V were each seen in 4% of stavudine/didanosine failures. Among the treatment arms, those starting with zidovudine/lamivudine/efavirenz had the lowest rate of failure but not the lowest rate of resistance mutations at failure (15 of 31, 48%). The lowest proportion of resistance mutations at failure was observed in the stavudine/didanosine/nelfinavir arm (22 of 77, 29%).

Treatment-Experienced Patients

Abacavir. The Zodiac study (CNA30021; Abstract 551) compared two regimens of abacavir/lamivudine/efavirenz in which abacavir was given either qd or bid. Virologic failure (> 50 copies/mL HIV-1 RNA) was observed in 10% in the abacavir qd arm and 8% in the bid arm to 48 weeks. Noninferiority of the abacavir qd

arm was previously presented. Of these only 31 of 70 (44%) had an HIV-1 RNA level sufficient for resistance testing (ie, > 400 copies/mL). Among these a non-significant trend toward greater numbers of mutations in the qd arm was observed. Among those with resistance data available 12 of 16 in the qd arm and 14 of 15 in the bid arm developed the K103N mutation. Also, in the qd arm 10 of 16 developed the M184V mutation compared with 5 of 15 in the bid arm.

Irbeck and colleagues (Abstract 661) compared the resistance profiles in 649 antiretroviral-naïve subjects with treatment failure of efavirenz/lamivudine with either abacavir or zidovudine (CNA30024). Virologic failure (consecutive plasma HIV-1 RNA levels > 50 copies/mL) was uncommon in this study (6% in the abacavir group and 4% in the zidovudine group). Post-failure genotypes were obtained in 13 of 20 in the abacavir and 10 of 13 in the zidovudine arms. Of these samples, 11 were wild-type and 12 had resistance mutations (NNRTI mutations in 12 of 12 samples and M184V in 10 of 12 samples). No TAMs were observed.

Didanosine. Clavel and colleagues (Abstract 670) explored the degree to which TAMs affect antiviral activity of and susceptibility to didanosine in a recombinant virus assay (AI454-176, Jaguar trial). Patients with stable on-treatment viremia were randomized to add didanosine ($n = 110$) to their therapy or not ($n = 58$). Genotypic and phenotypic predictors of week-4 change in HIV-1 RNA were evaluated. The distribution of didanosine fold changes at baseline was narrow, with only 23% and 13% of values above 2.0 and 2.5, respectively. Only a weak correlation was found between didanosine fold changes at baseline and week-4 change in HIV-1 RNA. However greater numbers of the specific mutations M41L, T215Y/F, L210W, K219Q/E, L74V, and T69D were associated with reduced virologic response ($P < .001$). The authors suggested that genotype may be a more useful predictor of in vivo didanosine activity.

Atazanavir. Colonna and colleagues (Abstract 656) examined the evolution of phenotypic and genotypic atazanavir resistance profiles in 100 treatment-

experienced subjects who received a variety of atazanavir-, atazanavir/r- or atazanavir/saquinavir-containing regimens. Of these 100 isolates, 18 developed the signature atazanavir mutation I50L. Over half of those developing I50L had resistance to only 1 or no other PIs at baseline. Among these 18 isolates the only major baseline PI mutation observed was the L90M. Mutations coemergent with I50L were L33V/F (in 5), E34K/Q/A (in 6), M36I/V/L (in 6), K45R (in 4), A71V/T/I (in 10), G73S/T (in 7) and V82A/F/T (in 3). Thus the emergence of I50L with atazanavir in treatment-experienced subjects is associated with a lack of PI cross-resistance at baseline.

Resistance to atazanavir by the I50L mutation. Weinheimer examined the resistance profiles and protease activity associated with the sentinel atazanavir mutation I50L in wild-type and mutant backgrounds (Abstract 625). Using the NL4-3 reference strain, the authors noted augmentation of I50L atazanavir resistance by the A71V mutation. The I50L mutant (+/- A71V) conferred fold changes of above 0.4 to all PIs except amprenavir. In isolates bearing A71V with either D30N, G48V, V82A, I84V, or L90M, the introduction of I50L was associated with general reductions in the levels of resistance to PIs with the exception of amprenavir, which was impacted only slightly. Protease activity in the absence of drug and measured as percent p24/p25 demonstrated that the I50L-A71V motif impaired protease activity in wild-type and mutant protease. Thus, I50L mutant (+/- A71V) appears to impair protease processivity and to enhance susceptibilities to most PIs, with more neutral effects being observed for amprenavir.

TMC114. Using a panel of 5,061 isolates, including 2,202 protease resistant isolates, De Mayer compared the susceptibilities to the PI TMC114 and compared these with those of approved PIs (Abstract 620). Isolates were grouped by the number of specific PI mutations: D30N, M46I/L, G48V, I50V, V82A/F/T/S, I84V, and L90M. Among isolates with 3 or more mutations the proportions susceptible (<4-fold change) to TMC114, amprenavir, saquinavir, atazanavir, and

lopinavir were approximately 55%, 21%, 22%, 17%, and 5%, respectively.

Fusion inhibitors. Enfuvirtide (T-20) is a synthetic 36-amino acid peptide corresponding to residues 127 to 162 of gp41. Enfuvirtide resistance is associated with changes in amino acids 36 to 45 of the HR1 domain. Xu studied the evolution of genotypic changes in the HR1 and HR2 domains (Abstract 659). Samples were derived from 17 highly treatment-experienced patients with virologic failure on an enfuvirtide-containing regimen. Mutations in HR1 were noted in all cases, including the previously unreported changes N42Q/H and N43Q. Continued evolution of HR1 mutations was observed. Six of 17 (35%) patients developed an S138A substitution in the HR2 domain after emergence of HR1 mutations (typically at position 43). Four patients demonstrated the loss of both HR1 and HR2 mutations following cessation of enfuvirtide therapy.

Monchetti evaluated clinical samples, at baseline and at follow-up, derived from 8 individuals with virologic failure on enfuvirtide-containing salvage therapy (Abstract 660). A modified NL4-3 recombinant bearing the test isolate's gp41 sequence spanning the HR1 and HR2 domains was used. In the recombinant phenotyping assay greater than 100-fold decrease in susceptibility (ie, resistance) from baseline was observed in 7 of 8 isolates after at least 9 months of therapy. Only 2 of 7 clinical isolates had greater than 50% reductions in drug-free replication capacity (RC) from baseline. Site-directed single mutations conferred significant resistance to enfuvirtide. However, such mutations conferred at most only modestly lower RCs (eg, 60% to 80% were observed with single changes at codons 43, 45, or 72).

Tenofovir resistance and K65R. The K65R mutation is the signature resistance mutation arising with tenofovir and may confer cross-resistance to all nRTIs except zidovudine and stavudine. Several presentations focused on the characteristics of this mutation (Abstracts 54, 55, 626, 627, 637).

Amiel noted the prevalence of K65R mutants in 24 of 3025 (0.8%) samples

sequenced within a French database covering the last 5 years (Abstract 627). M184V was present in 8 (44%) of these samples. The Q151M mutation associated with multi-nRTI resistance was seen in 9 (50%) samples. By comparison, the overall prevalence of Q151M in this database was 33 of 3025 (1%); thus, among samples with Q151M, 9 (44%) had K65R concurrently. The authors comment that the overall rarity of the K65R and Q151M mutations alone, but relatively high co-occurrence, raises concerns for coselection and represents a potential pathway for cross-resistance distinct from TAMs. The prevalence of K65R is likely to increase with the widespread use of tenofovir.

The impact of K65R on viral fitness was evaluated using 4 primary isolates in which this mutation arose during tenofovir therapy (Abstract 637). Growth was compared with pretherapy isolates and to 2 reference strains. Comparative growth curves in the setting of increasing tenofovir concentrations demonstrated the relative growth advantage of K65R isolates over pretherapy isolates. However, competition cultures in a drug-free environment demonstrated the marked growth impairment of K65R isolates relative to both the control isolates and baseline isolates. These data confirm prior observations that K65R impairs viral fitness.

Two presentations focused on mechanistic aspects of K65R as a resistance mutation. White demonstrated that compared with wild-type enzyme, a reverse transcriptase with K65R exhibited both decreased incorporation of tenofovir, abacavir, stavudine, and zidovudine (5- to -17-fold less) and variably decreased excision of incorporated drug (Abstract 55). This reduced excision effect was most apparent for zidovudine, being 43% for wild-type and 19% for the K65R mutant in the presence of the next appropriate nucleotide. The authors suggested that for a given drug the relative interactions of diminished binding and excision in the setting of K65R are responsible for the observed phenotypes.

A complementary study by Parikh and colleagues (Abstract 54) observed that the frequency of K65R increased from 0.8% to 3.8% from 1998 to 2003 among 65,000 samples in a commercial

database. Within this dataset a negative correlation was observed between K65R and the TAM cluster M41L/L210W/T215Y. In vitro, recombinant isolates bearing K65R were 2.5- to 10-fold resistant to nRTIs tested except those with a 3'azido moiety (eg, zidovudine), which demonstrated wild-type sensitivity. Furthermore recombinants with M41L/L210W/T215Y or 67N/70R/T215F/K219Q with K65R demonstrated reduced zidovudine resistance (approximately 3-fold with K65R versus greater than 30-fold without K65R) and also reduced primer unblocking (wild-type activity with K65R versus approximately 10-fold without K65R). Thus K65R was demonstrated to antagonize both the mechanism and expression of zidovudine resistance. These data may help explain the observed low rate of concurrent selection of TAMs and K65R.

Other Factors

Hypersusceptibility. ACTG 368 compared the activities of efavirenz/indinavir/abacavir and efavirenz/indinavir in nRTI-experienced, PI-naïve subjects (Abstract 669). NNRTI-experienced subjects ($n=26$) received open label efavirenz/indinavir/abacavir. NNRTI-naïve subjects received efavirenz/indinavir with abacavir ($n=140$) or without abacavir ($n=143$). At week 16 there were no differences in the rates of virologic failure in abacavir and no-abacavir arms, being 27% and 31%, respectively ($P=0.5$). Within this study Demeter and colleagues evaluated the impact of baseline drug resistance on 16-week virologic outcomes. The phenotypic susceptibilities were measured in a multiple cycle assay. The impact of specific baseline reverse transcriptase mutations previously associated with efavirenz hypersusceptibility, including T215F/Y, D67N, H208Y, and L210W, was also evaluated. At baseline, 37% of subjects had a greater than 3-fold change in susceptibility to abacavir. Neither the baseline abacavir susceptibility nor the overall phenotypic susceptibility score were predictive of virologic failure. However, virologic failure was significantly less common if 2 or more efavirenz hypersusceptibility mutations were present ($P=0.0017$). Notably, in a small number of individuals on indinavir/efavirenz

only, the L74V reverse transcriptase mutation was coselected with K103N and L100I.

Haubrich and colleagues (Abstract 671) evaluated the impact of baseline hypersusceptibility to delavirdine in representative samples from 96 subjects in ACTG 359. This study evaluated the following regimens in 185 nRTI-experienced subjects: delavirdine/saquinavir sgc with either ritonavir, ritonavir/adefovir, nelfinavir, or nelfinavir/adefovir. Virologic response, evaluated at weeks 4 and 16, was defined as a plasma HIV-1 RNA at or below 500 copies/mL. In all models, the entry plasma HIV-1 RNA level was predictive of virologic response. Delavirdine hypersusceptibility defined by a cutpoint 0.4 fold or lower, or on a continuous scale, was predictive of virologic success at week 4 but not week 16. Using data from a logistic regression model, the authors suggested that the optimal cutpoint for delavirdine hypersusceptibility is 0.3- to 0.4-fold or lower.

CXCR4 Coreceptor studies. HIV that utilizes the CXCR4 coreceptor (when present, the virus is termed X4 virus), or that induces syncytia (SI virus), has been associated with accelerated progression of HIV disease. Jensen and colleagues (Abstract 415) utilized previously described position-specific scoring matrices (PSSM) to assess V3 sequences for both X4 and SI potential. They applied this matrix to early samples from 32 seroconverters in the MACS to evaluate whether rapid progressors are characterized by early acquisition of X4/SI virus. Twenty-one of 32 were rapid progressors and 3 of 21 had X4 virus, 2 of these being dually infected compared with 0 of 11 standard progressors. A correlation was noted between preinfection CD4+ counts and PSSM score ($P=0.031$). Mean and maximum SI scores were also higher in rapid progressors ($P<0.016$). Analyses suggested that mean SI score influenced progression independently of dual infection ($P=0.027$).

Mosier (Abstract 409) described multiple independent coreceptor switch variants that were selected from 6 R5 (ie, virus containing the CCPR coreceptor) parental strains after 12 to 120 days of culture in mixtures of cells expressing

CCR5 or CXCR4. As these viruses transitioned to X4, the susceptibility to CCR5 and to CXCR4 inhibitors increased, suggesting less efficient coreceptor use as an obstacle to switching. Further, replication was less efficient and infectivity was impaired for switch variants, suggesting loss of fitness as a further obstacles to switching.

UK-427,857 is a novel CCR5 antagonist in development. A concern regarding in vivo use of R5 antagonists is the possible selection for X4 variants during treatment. Westby described a patient harboring distinct R5, X4, and dual-tropic variants when inadvertently enrolled in a phase IIa, 10-day monotherapy study (Abstract 538). The patient experienced no drop in plasma HIV-1 RNA despite measurable drug exposure and ex vivo UK-427,857 CCR5 receptor occupancy within the anticipated range. Clonal sampling showed a shift from a mixture of viruses using R5 and R5X4 coreceptors at baseline to a mixture of viruses using X4 and R5X4 at day 11. Reversion to R5 predominance was observed after UK-427,857 was stopped.

The potential clinical relevance of X4 tropic viruses was highlighted in a presentation from Solomon (Abstract 654). Eleven subjects had on-treatment viremia over at least 12 months; 5 had declining and 6 had rising CD4+ counts. All individuals had at least 1 major mutation in protease or reverse transcriptase associated with resistance. Thymic growth of lymphocyte derived isolates was equivalent between the 2 groups. However replication in lymphocytes and CD4+ depletion was greater in isolates derived from nonresponders ($P<0.05$). Further, for infected and uninfected CD4+ T-cells, apoptosis was greater by isolates derived from nonresponders ($P<0.001$). X4 tropism was demonstrated in 4 of 5 non-responders and 2 of 6 responders. These data underline the potential relevance of X4 tropism in the clinical setting.

Observational cohort studies. Hogg and Harrigan (Abstracts 674, 689) described the prevalence of drug resistance among subjects followed up in British Columbia between August 1996 and September 2000 (The Homer Cohort). Within this cohort of 1,388 individuals,

the median follow-up time was 52.7 months. Genotypic evidence of drug resistance was observed in 393 individuals; resistance to lamivudine, other nRTIs, NNRTIs, and PIs was seen in 68%, 35%, 50%, and 27% of subjects, respectively. During follow-up, 238 subjects died (crude mortality rate, 17.2%); among these individuals, resistance to 1, 2, or 3 classes, to lamivudine, to NNRTIs, to other nRTIs; or to PIs was seen in 15%, 13%, 1%, 19%, 18%, 8% and 3%, respectively. No resistance was seen in 71% of subjects who died. Among those with a minimum follow-up time of 12 months and using multivariate analyses that controlled for other contributory variables, individuals with NNRTI resistance had death rates that were 2.74 times higher (range, 1.55-4.84; $P < 0.001$) than those without such resistance. This association should be interpreted cautiously.

Fessel analyzed 3,320 sequences from 2,324 persons in Northern California who had genotypic resistance testing between 1998 and 2002 (Abstract 690). Outcomes after resistance testing were analyzed. Eighty-two subjects (3.5%) had 3-class resistance (intermediate or high-level resistance to all 18 FDA-approved HIV drugs/formulations). In 324 (13.9%), 2-class resistance was detected (intermediate or high-level resistance within 2 drug classes with complete susceptibility to a third class). Of this group 74 (23%) had PI and nRTI resistance. Three-class resistance occurred almost exclusively in those with more than 4 years of prior therapy. Among those with 3-class resistance, sustained or transient virologic responses to salvage therapy were seen in only 10% and 18%, respectively. Among those with PI and nRTI resistance introduction of an NNRTI-based regimen reduced plasma HIV-1 RNA to below 50 copies/mL in 53%. This was sustained in only 36% of these individuals among whom NNRTI-associated resistance emerged on viral rebound.

Low-Level Viremia and Viral Persistence

Various studies evaluated the relevance of preexisting minority variants to ongoing therapy (Abstracts 37, 39, 57). Mellors and colleagues (Abstract 39)

evaluated the relevance of preexisting minority variants to treatment failure associated with efavirenz resistance. The 11 NNRTI-naive and 12 NNRTI-experienced subjects were enrolled in ACTG 398 and did not have NNRTI mutations at entry by standard sequencing techniques. Single genome sequencing demonstrated baseline NNRTI mutations in 6 of 11 NNRTI-experienced patients with the following frequencies per positive patient: Y181C and G190A (5/15 sequences); Y181C (3/19); Y181C (3/22); V108I (2/35); K103N (1/33); and K103N (1/34). By comparison, NNRTI-resistant variants were found in only 2 of 12 NNRTI-naive patients: L100I and P225L (1/33 sequences each) and K103N (1/41 sequences). In 5 of 6 NNRTI-experienced patients phylogenetic analyses showed clustering of the baseline and failure of NNRTI-resistant variants. In the 2 NNRTI-naive patients, the baseline K103N variant clustered with the failure genotype but the L100I and P225L variants did not. These various studies supported the concept that in the absence of drug pressure, preexisting mutants may continue to circulate below the limits of detection by current assays.

Martin (Abstract 653) described correlates of CD4+ cell count changes over time in 47 subjects on stable antiretroviral therapy, with stable plasma HIV-1 RNA of at least 100 copies/mL for at least 12 months and with resistance to at least 1 drug, in the Study of the Consequences of the Protease Inhibitor Era (SCOPE). At baseline, the median CD4+ count, plasma HIV-1 RNA, number with PI resistance, and follow-up time were 340 cells/ μ L, 3.5 log₁₀ copies/mL, 40 of 47 subjects, and 13.2 months, respectively. The median proportion of CD8+ cells that were CD38+/HLA-DR+ was 18.6% (range, 5%-50%). Repeated Measures Regression outcome modeling demonstrated that CD4+ cell change was a function of time and baseline proportion CD38+/HLA-DR+ CD8+ with decreases in CD4+ counts over time associated with higher proportions of CD38+/HLA-DR+ CD8+.

In a related presentation (Abstract 453), Karlsson and colleagues evaluated subjects with stable on-treatment plasma HIV-1 RNA values as follows: sup-

pressed below 50 copies/mL ($n = 13$), suppressed but with episodes of non-sustained viremia ("blips"; $n = 15$), or sustained viremia in the range 50 to 1000 copies/mL ($n = 18$). HIV-specific T-cell immunity was measured using interferon (IFN)-gamma ELISPOT assay. T-cell activation was defined by CD38+ and HLA-DR coexpression. The median CD4+ count in the 3 groups was 674 cells/ μ L, 496 cells/ μ L, and 460 cells/ μ L, respectively. The median duration of this stable plasma HIV-1 RNA pattern preentry was 30 months, and subjects had a median of 27 months' follow-up on study. More than 50% of those with plasma HIV-1 RNA in the range of 50 copies/mL to 1000 copies/mL had HIV-1 RNA values above 1,000 copies/mL at 30 months. This was significantly shorter than the other 2 groups, where such failure was rare. Further the levels of HIV-specific T-cell immunity and T-cell activation were greater in breadth and magnitude in subjects with either intermittent or persistent low-level viremia compared with subjects with sustained viral suppression. Also those with persistent low-level viremia had increases in immune activation relative to those with blips. This group also had significant increases in PI ($P = 0.004$) and nRTI ($P = 0.005$) resistance over time. These data highlight negative aspects of persistent viremia in the range 50 copies/mL to 1,000 copies/mL relative to sustained suppression or blips. The authors suggested that therapy modification in the setting of stable viremia in the range 50 to 1000 copies/mL merits consideration.

Kieffer and colleagues (Abstracts 650, 651) reported the results of 2 studies evaluating genotypic evidence of resistance in those with stably suppressed plasma viremia. Abstract 651 was a study of plasma HIV-1 RNA estimates performed 3 times weekly for 12 weeks in 10 subjects with stable on-treatment suppression of the plasma HIV-1 RNA below 50 copies/mL for at least 6 months. Viral loads were run concurrently in 2 laboratories with the Roche Amplicor Monitor (1.5). So-called blips were observed in 9 of 10 subjects (mean, 2 blips/patient over 12 weeks). Only 2 blips lasted more than 96 hours. Defining a blip as a value above 150 HIV-1 RNA copies/mL, any quantifiable

value that was positive from both laboratories, or consecutive quantifiable values reduced the total number of blips from 20 to 4, and these were observed in only 2 patients. Clonal analysis of the plasma HIV reverse transcriptase was successful in 9 of 10 subjects with an average of 3 clones per patient at each time point. Resistance mutations were observed in 8 of 9 subjects at baseline but no evolution of resistance was observed. The authors contended that blips are common but often nonreproducible and not necessarily associated with evolution of preexisting drug resistance.

Fitness and Replication Capacity

Barbour (Abstract 388) described the HIV RCs in 191 recently infected antiretroviral-naïve individuals. Using genotyping, 168 isolates were wild-type, 7 were PI resistant, 13 were only-nRTI resistant, 11 were only-NNRTI resistant, and 4 were only-nRTI and NNRTI resistant. Partial correlation coefficients of these resistant groupings (PI, nRTI, and NNRTI) to RC were 3.6%, 0.5%, and 1.7%, respectively, suggesting that the contributions of resistance motifs to RC were relatively modest. Isolates with PI mutations had significantly lower average RCs than wild-type isolates ($P=0.01$). Split-regression analyses showed that isolates with RCs below 43% had higher CD4+ counts ($P=0.004$). Further, a nonsignificant trend was observed such that on suppressive therapy for 18 months those with RCs below 43% averaged greater CD4+ count gains compared with those without.

Koh and colleagues (Abstract 634) evaluated the relative growth impact of specific *gag* cleavage site mutations E12K, L75R, H219Q, V390D, R409K, and L449E, and protease mutations L10F, V32I, M46I, I54M, A71V, and I84V derived by serial passage with amprenavir. A clone bearing only the PI mutations failed to replicate. Clones bearing combinations of these *gag* mutations replicated equivalently to wild-type clones and were as susceptible to amprenavir. Further, isolates bearing only these *gag* mutations passaged in the presence of amprenavir acquired amprenavir resistance mutations more

rapidly than the wild-type virus. Thus *gag* mutations may impair replication but may also impact pathways to PI resistance.

Hu (Abstract 638) described the relative fitness patterns of site-directed mutants bearing the reverse transcriptase mutations T215F/Y in backgrounds of M41L, M41L/L210W, and M41L/D67N/L210W in the presence and absence of zidovudine. In all matched backgrounds the T215F recombinants replicated less efficiently than the T215Y mutant. The authors suggested these observations may explain the more frequent observation of the T215Y clusters.

Using data derived from drug-sensitive HIV-1 in the ViroLogic commercial database, Bates and colleagues evaluated associations between changes in the C-terminal 83 codons of HIV *gag* and differences in replication capacities (Abstract 121). Observed changes included lower replication capacities in isolates with mutations at codon 484 ($P=0.0019$) and higher replication capacities in isolates with mutations 418 and those with insertions at codon 458—the so-called PTAPP motif. The authors speculated that such insertions at codon 458 may increase the efficacy of p6-Tsg101 binding, resulting in enhanced budding and higher replication capacity.

Resistance Associations by Genotype Database Analyses

Using data from 4907 genotypes at a commercial database, Flandre selected those with TAMs, or E44D and V118I exclusively, and evaluated mutations clustering by number of mutations (Abstract 645). Significant clustering of M41L/L210W/T215Y and D67N/K70R/T215F/K219E/Q was observed, with the former predominating when more than 3 mutations were present. Resistance to zidovudine and stavudine was also greater with the M41L/L210W/T215Y cluster. The most common single mutations were M41L, K70R, V118I, and T215Y, with the greatest level of resistance associated with the T215Y mutation.

Kagan employed a variety of statistical models to define associations between known resistance mutations

and other changes in codons 1 to 400 of the HIV reverse transcriptase (Abstract 629). The 28,655 samples with genotypic resistance to a protease inhibitor or reverse transcriptase inhibitor were obtained between January 2002 and June 2003 and sequences stored at a commercial database. Three previously defined nRTI codon clusters (with associated novel codons) were observed as follows: previously defined, 41, 44, 118, 210, 215 (associated novel, 39, 43, 203, 223); 67, 69, 70, 219 (218, 228); and 62, 65, 68, 75, 77, 116, 151. Three previously defined NNRTI codon clusters (with associated novel codons) were observed as follows: 101, 108, 181, 190 (221); 106, 179, 188 (227); 100, 103, 225 (238).

International Studies on Resistance

HIV-2 strains are known to possess intrinsic resistance to FDA-approved NNRTIs. Reid described the nRTI resistance profiles of HIV-2 strains using primary isolates and reference strains with multiple cycle assays in MT4 cells (Abstract 691). Sequence homology between the various HIV-1 and HIV-2 strains tested was estimated at 64%. When HIV-2 strains were passaged with zidovudine selection of typical zidovudine mutations was observed uncommonly; when passaged in the presence of zidovudine/didanosine, few mutations were selected, including K65R, M184I, D67N, and H221Y. Phenotypic studies suggest a relatively greater intrinsic resistance of HIV-2 (compared with HIV-1) to zidovudine (ranging from 2.2- to >200-fold greater) but not to didanosine. Further, HIV-2 isolates had relatively efficient replication at high zidovudine concentrations.

Fleury and colleagues (Abstract 688) described the phylogenetic profiles of HIV isolates from antiretroviral-naïve subjects at 2 international sites. In Abidjan, Cote D'Ivoire ($n=206$), the relative subtype prevalence was CF02_AG > A > CRF06_cpx, CRF04_cpx. The protease polymorphism M36I was observed in 94% of CF02_AG samples. Mutations in HIV-1 associated with resistance were observed in 5.6% of samples. In Ho Chi Min city, Vietnam ($n=200$), the CRF01_AE was the most predominant subtype. TAMs and the PI

mutations D30N and L90M were observed but rarely. Mutations associated with resistance in HIV-1 were observed in 6.5% of samples.

Hall and colleagues (Abstract 694) compared outcomes by clade B or C infection in the 1216 individuals enrolled in the 2NN study. This international study evaluated the 48-week outcomes in drug-naive individuals treated with distinct nevirapine- and efavirenz-based regimens, each in combination with stavudine and lamivudine. Comparison was made between 174 randomly chosen participants and 102 with virologic failure (ie, failure to reach or to sustain an HIV-1 RNA below 50 copies/mL). More virologic failures than randomly selected patients were subtype C, with 48% and 36%, respectively. Also more of the virologic failures were from South Africa than the randomly selected patients: 56% and 36%, respectively. Treatment emergent mutations K103N and V106M were more common in those with subtype C or on efavirenz. Among those with baseline resistance, 12 of 13 experienced virologic failure. Notably, K65R was observed in 8 of 119 subjects whose nRTI therapy was stavudine/lamivudine, supporting recent observations that this mutation can emerge on stavudine-based therapy.

A presentation by Aluoch (Abstract 580) noted a failure to define any known resistance mutations in 34 HIV-1 subtype C isolates from 34 treatment naive individuals in South Africa. Some polymorphisms were seen more frequently in subtype C than subtype B HIV-1, including E36A (72% vs 0%, respectively), T39E/D (93% vs 0%), K173A/T (100% vs 0%), and in protease L89I/M (97% vs 0%).

A complementary presentation by Calazans (Abstract 692) suggested that the L89M change in subtype F protease may impact resistance to PIs. L89M site-directed mutants were constructed using protease-susceptible clones of subtype B and F. By MT4 cell-MTT cell viability assay, the L89M mutation conferred 6.2-, 5.6-, 4.7-, 4.5-, 3.4-, and 2.0-fold increases in EC_{50} to nelfinavir, indinavir, ritonavir, amprenavir, lopinavir and saquinavir, respectively. The clinical relevance of these changes with respect to treatment failure with non-

clade B strains merits further evaluation.

Torimiro and colleagues (Abstract 223) evaluated HIV diversity among 41 HIV seropositive rural rainforest dwellers in Cameroon. The prevalence of HIV was 3.1%. Phylogenetic classification of protease-RT sequences showed that 95% were recombinants, with many novel strains. Limited full-length sequencing of 11 isolates showed that most contained partial sequences from subtypes A and E. The ongoing evolution of this locally mature epidemic has relevance to regions where the epidemic is more recent and for vaccine strategies.

Petroni and colleagues (Abstract 693) evaluated the prevalence of HIV recombinants among 316 Argentinean individuals whose samples were evaluated at a reference laboratory between June 1999 and February 2002. Cross-sample phylogenetic comparisons were made of the HIV protease and the first 960 bases of the RT. The observed distribution of subtypes was B 51.9%, B/F recombinant 47.8%, and F 0.3%. The B/F recombinant was significantly more common among women ($P < 0.001$) and children ($P < 0.001$). Among isolates with protease mutations at codons V82A/F/T, the changes K20R/M and I54V/L were observed more frequently among B/F recombinants.

Pharmacology

nRTI

Gries and colleagues presented sub-study results from a randomized clinical trial of ribavirin and either pegylated interferon alfa-2a or interferon alfa-2a in the management of hepatitis C virus infection in HIV-infected patients (Abstract 136). They examined the intracellular and plasma pharmacokinetics of zidovudine, lamivudine, and stavudine in patients before and after receiving ribavirin and peginterferon alfa-2a for 8 to 12 weeks. The plasma AUC_{0-12hr} of zidovudine, lamivudine, and stavudine were not altered after the addition of ribavirin and peginterferon alfa-2a, nor were the intracellular AUC_{0-12hr} values of their active triphosphorylated forms.

Kearney and colleagues investigated the pharmacokinetics of tenofovir in patients with hepatic impairment and in patients receiving therapy for viral hep-

atitis (Abstract 600). Tenofovir pharmacokinetics were similar among patients with severe hepatic impairment ($n = 8$), moderate impairment ($n = 7$), and unimpaired controls ($n = 8$) as defined by the Child-Pugh-Turcotte system. They also examined the single-dose pharmacokinetics of adefovir and ribavirin with and without tenofovir, and found no significant interactions.

Kaul and colleagues evaluated the pharmacokinetics of extended-release stavudine with and without tenofovir in 18 HIV-seronegative subjects (Abstract 602). They did not find a significant change in the C_{max} , AUC, or median time to maximal concentration in stavudine when coadministered with tenofovir.

Efavirenz

Taylor and colleagues evaluated efavirenz concentrations in 8 patients for 3 weeks after discontinuation of the drug (Abstract 131). The indication for stopping efavirenz included virologic failure, toxicity, change of dual NNRTI to single nRTI, or treatment interruption after seroconversion. They also evaluated 25 other patients who were interrupting antiretroviral therapy post seroconversion and stopped efavirenz 5 to 7 days prior to stopping nRTIs. They found significant plasma levels of efavirenz 2 weeks after discontinuation. Although they did not detect any new resistance mutations in patients stopping efavirenz 7 days prior to stopping nRTIs, they concluded that efavirenz should be discontinued 2 weeks prior to stopping nRTIs, or that it should be exchanged for another antiretroviral medication with a shorter half-life prior to interrupting therapy.

Ribaudo and colleagues presented data on efavirenz pharmacokinetics from a substudy of ACTG 5095 (Abstract 132). They found that race was significantly related to clearance of efavirenz: white, non-Hispanic subjects had a 32% faster clearance than black or Hispanic subjects. There was also some evidence that drug discontinuation was related to decreased clearance ($P = 0.052$) and increased C_{max} ($P = 0.048$). These parameters were not related to virologic response or rates of a first central nervous system toxicity. Authors from the same study, Haas and colleagues,

offered an explanation for this differential rate of clearance (Abstract 133). They linked decreased clearance of efavirenz to a common allelic variant at CYP2B6, the enzyme mainly responsible for metabolizing efavirenz. The variant was more common among blacks (20%) than whites (3%) and was associated with a 3-fold-higher plasma efavirenz level.

Hitti and colleagues examined the association of sex and weight with the pharmacokinetics of efavirenz, indinavir, and nelfinavir with data collected from 6 different ACTG trials (Abstract 604). They found no association of sex with nelfinavir, M8 (an active metabolite of nelfinavir), or indinavir levels. However, they found a significantly lower efavirenz AUC in women compared with men. This is in contrast to the study by Ribaud, which did not find an association between sex and efavirenz AUC. They also examined the association of weight with the pharmacokinetics of these drugs. Increased weight reduced the AUC of efavirenz and indinavir.

Gerber and colleagues presented the results of ACTG 5108, which evaluated the effect of efavirenz on simvastatin and atorvastatin levels in 27 HIV-seronegative individuals (Abstract 603). Efavirenz reduced the AUC₂₄ of simvastatin by 58%, and atorvastatin by 43%. This suggests that higher doses of simvastatin and atorvastatin may be necessary to achieve the desired effects on plasma lipid levels.

Drug-Drug Interactions

Triple protease inhibitors. Boffito and colleagues presented data on the pharmacokinetics of saquinavir (hard gel formulation) administered with low-dose ritonavir, and given with and without atazanavir (Abstract 607). Saquinavir 1600 mg was given with ritonavir 100 mg for 1 dose to 20 HIV-infected participants. On day 2, the same doses were continued each day along with atazanavir 300 mg qd for 30 days. There were no discernable changes in plasma lipids after addition of atazanavir. There was a significant increase in plasma bilirubin levels. They found that administration of atazanavir increased the C_{trough}, C_{max}, and AUC by

60%, 42% and 112%, respectively. The authors postulated that atazanavir may boost saquinavir levels by a mechanism distinct from that of ritonavir.

ACTG 5143 showed a significant decrease in both lopinavir and amprenavir levels when fosamprenavir and lopinavir/ritonavir were administered together. Corbett and colleagues presented the results of 2 strategies to overcome this interaction, neither of which were satisfactory (Abstract 611). Eleven HIV-seronegative subjects received lopinavir 400 mg/ritonavir 100 mg plus 700 mg of fosamprenavir bid for 7 days. Then they received the same doses separated by 4 hours for 7 days. After this, they received lopinavir 800 mg/ritonavir 200 mg qd and fosamprenavir 1400 mg qd separated by 12 hours. Compared with simultaneous administration, the 2 separation strategies resulted in significantly higher lopinavir levels, but amprenavir levels remained suboptimal.

A second study presented by Wire and colleagues also evaluated the interaction of fosamprenavir and lopinavir/ritonavir (Abstract 612). They tried 2 dosing strategies to improve drug levels of amprenavir and lopinavir. 36 subjects received lopinavir 400 mg/ritonavir 100 mg bid or amprenavir 600 mg/ritonavir 100 mg bid as the control treatment. In the first study, subjects received fosamprenavir 1400 mg bid plus lopinavir 533 mg/ritonavir 133 mg. Thirteen of 36 subjects dropped out early, mostly due to side effects. In the second study, subjects received lopinavir 400 mg bid/ritonavir 200 mg bid, and fosamprenavir 700 mg bid. Sixteen of 36 dropped out early, mostly due to side effects. Both studies resulted in lopinavir levels that were higher than when subjects received lopinavir/ritonavir alone. However, amprenavir levels were significantly reduced in both studies compared with receiving fosamprenavir/ritonavir alone. The authors concluded that the suboptimal amprenavir levels coupled with the high rate of side effects limited the utility of these dosing strategies.

Vezina and colleagues followed up 12 HIV-infected subjects on lopinavir 400 mg/ritonavir 100 mg bid along with amprenavir 600 mg bid (Abstract 609). After 2 weeks, pharmacokinetic sampling was performed. Dose adjustments

were made to assure drug levels consistent with manufacturer's recommendations. Six of 12 patients required increased doses of lopinavir/ritonavir (2 received 533 mg/133 mg and 4 received 667 mg/167 mg). Three of 12 participants required an increase of amprenavir (2 received 750 mg and 1 received 900 mg). No adverse events were noted. The authors concluded that the interaction between these drugs was difficult to predict and dose individualization through pharmacokinetic monitoring may be indicated.

Tenofovir/didanosine. Dose reduction for didanosine when co-administered with tenofovir has previously been recommended due to increased didanosine levels in the presence of tenofovir and an increased rate of pancreatitis. Clotet and colleagues (Abstract 749) raised concerns for a potential toxicity associated with combination tenofovir and full-dose didanosine (400 mg qd). In a retrospective database analysis, 150 subjects with plasma HIV-1 RNA below 50 copies/mL switching to tenofovir/didanosine were compared with a similar group of 152 subjects switching to either full-dose didanosine or tenofovir. After changing therapy, subjects maintained suppression of plasma HIV-1 RNA. More than 50% of subjects in the tenofovir/didanosine group had reductions of more than 100 CD4+ cells/ μ L, and up to 30% of this group lost more than 200 cells/ μ L, at last follow-up ($P \leq 0.05$). By comparison, 85% to 90% of subjects in the tenofovir or didanosine groups, respectively, had CD4+ counts that were unchanged or had increased. Eight subjects on tenofovir/didanosine underwent a didanosine dose reduction to 250 mg/day; 3 months after this the mean increase in CD4+ count was 60 cells/ μ L. The authors raised concerns for a potential toxicity associated with full-dose didanosine in combination with tenofovir.

Lopinavir and saquinavir. Dam evaluated the in vitro antiviral activity of a variety of fixed-molar combinations of lopinavir and saquinavir against wild-type and mutant HIV strains in a single-cycle cell-indicator assay (Abstract 622). Enhancement of saquinavir activity by

lopinavir was observed in isolates with low-level saquinavir resistance but high-level lopinavir resistance ($P=0.0004$). Contrary to prior reports, synergism was not observed with wild-type isolates.

Mother-To-Child Transmission of HIV

Single-dose nevirapine has gained acceptance as a simple, inexpensive, and effective intervention to decrease mother-to-child transmission (MTCT) of HIV. Previous studies have documented a high rate of NNRTI-resistant virus in mothers in the immediate post-partum period. Access to antiretroviral therapy in the developing world is growing, and NNRTI-based regimens will likely be used often. The impact of NNRTI resistance on these women's future responses to NNRTI-based therapy has not previously been reported.

Martinson and colleagues presented a large study of 623 South African women receiving single-dose nevirapine for prevention of MTCT (Abstract 39). Overall, 38.2% of women and 42.4% of their infants had NNRTI resistance in the postpartum period. The rate of MTCT was 8.6%. Nevirapine resistance was associated with MTCT in a univariate analysis, but this was not evident after controlling for maternal viral load and CD4+ cell count. Nevirapine resistance in the mother was related to lower CD4+ cell counts, higher plasma HIV RNA levels, shorter time from delivery to genotypic testing, and receiving more than 1 dose of nevirapine prior to delivery (women received extra doses of nevirapine in some cases, eg, for false labor or extremely prolonged labor).

Lallemant and colleagues evaluated single-dose nevirapine in combination with zidovudine for prevention of MTCT (Abstract 40). All women in this randomized, double-blind, placebo-controlled trial received open-label zidovudine starting in the third trimester. The women were randomized to receive either nevirapine at delivery, and nevirapine for the infant, nevirapine at delivery and placebo for the infant, or placebo at delivery and placebo for the infant. The placebo-placebo arm was discontinued after the first interim analysis. At that time, the MTCT rate for placebo-placebo was 6.3% (95% confi-

dence interval [CI], 4.2-9.5%) compared with 1.1% (95% CI, 0.4-3.0%) for nevirapine-nevirapine. The final results for the nevirapine-nevirapine and nevirapine-placebo arms were 2.0% (95% CI, 1.2-3.4%) and 2.8% (95% CI, 1.8-4.4%), respectively, which met the protocol definition for noninferiority.

Chalermchokcharoenkit and colleagues presented results from an open-label study of 220 HIV-infected pregnant women who were given zidovudine starting at 34 to 36 weeks, followed by an intrapartum single dose of nevirapine (Abstract 96). Infants were given a single dose of nevirapine and 2 weeks of zidovudine after birth. They found that 10 of 223 infants (4.6%; 95% CI, 2.5%-8.6%) became HIV-infected, and 5 of 10 were HIV RNA PCR-positive at birth. One month postpartum, 17% and 2% of women had nevirapine and zidovudine resistance, respectively. Two of 10 HIV-infected infants had nevirapine resistance. This regimen was well tolerated, and anemia was the most common adverse event.

Jourdain and colleagues presented the results of an open-label study of 255 women starting a first nevirapine-based antiretroviral regimen (Abstract 41). Forty-two were not previously exposed to nevirapine, and 213 had received single-dose nevirapine during pregnancy to prevent MTCT. Sixty-three of 213 women exposed to nevirapine were previously found to have had genotypes associated with NNRTI resistance from samples obtained 2 weeks postpartum. The median baseline CD4+ counts were 169 cells/ μ L and 182 cells/ μ L in the nevirapine-exposed and unexposed groups, respectively, and the mean plasma HIV-1 RNAs were 4.61 \log_{10} copies/mL and 4.51 \log_{10} copies/mL, respectively. In the nevirapine-unexposed group, 75% had fewer than 50 HIV-1 RNA copies/mL 6 months after starting the nevirapine-based regimen, compared with 53% of the nevirapine-exposed women who did not have genotypic evidence of resistance postpartum and 34% of the nevirapine-exposed women who did have genotypic evidence of resistance. This implies women receiving single-dose nevirapine have an increased rate of virologic failure during subsequent NNRTI-based regimens.

Shapiro and colleagues presented

results on behalf of the PACTG regarding mode of delivery and risk of MTCT (Abstract 99). They focused their analysis on the role of elective caesarian section in women who had plasma HIV RNA levels below 1000 copies/mL. The overall risk of MTCT was 0.7% in this group. After controlling for known risk factors for MTCT (plasma HIV RNA levels, use of multidrug antiretroviral therapy, maternal CD4+ cell count, etc), they did not find a benefit of elective caesarian section, but did find a benefit of multidrug antiretroviral therapy even when having such a low plasma HIV RNA.

International Studies

South Africa

Churchyard and colleagues summarized an antiretroviral therapy program for gold miners in South Africa (Abstract 2). The prevalence of HIV infection in this population is estimated at 28%. The gold-mining company began a program in August 2002 to provide antiretroviral medications for those in need via a comprehensive, standardized delivery system. The first-line regimen was zidovudine/lamivudine/efavirenz, and the second-line regimen was didanosine/abacavir/lopinavir/ritonavir.

Overall, 1222 employees were eligible for antiretroviral therapy and 1098 (90%) started therapy. In 305 (28%), adverse events occurred, including 17 (1.6%) grade 4 events. There were 37 (3.4%) deaths after starting antiretroviral therapy. Retention was excellent, with 92% remaining in the antiretroviral therapy program. The median baseline CD4+ count was 145 cells/ μ L, and this nearly doubled 6 months after starting antiretroviral therapy. At 6 months, 60% had an HIV RNA level below 50 copies/mL. Interestingly, participation of gold miners in the voluntary counseling and testing program increased significantly during the same time period, highlighting the link between access to antiretroviral therapy and willingness to be tested for HIV.

Mozambique

Palombi and colleagues presented the results of the DREAM Project, which has enrolled 802 adults (including 510

women) and 215 children since February 2002 (Abstract 148). Approximately one-half of adults and one-third of children have started antiretroviral therapy (zidovudine or stavudine, plus lamivudine and nevirapine). The lost-to-follow-up rate was 9.3% in adults and 3.3% in children. The proportion of adults with a CD4+ count below 200 cells/ μ L decreased from 68.2% at baseline to 22.4% after 1 year of antiretroviral therapy. Approximately 75% achieved and maintained an HIV RNA level below 50 copies/mL at 1 year. Among the children, the median decline in HIV RNA was 5.2 log₁₀ copies/mL and the CD4+ cell percentage increased by a median of 10.4 percentage points. The death rate was 12.5% and 11.5% in adults and children, respectively.

India

Patel and colleagues presented results from a clinical cohort from Ahmedabad and Pune, India (Abstract 584). They compared the CD4+ cell count responses to efavirenz- (n=254) or nevirapine- (n=254) based regimens. The median baseline CD4+ counts were 100 cells/ μ L and 115 cells/ μ L, respectively. The median CD4+ counts after 1 year of starting antiretroviral therapy increased by 259 cells/ μ L and 213 cells/ μ L, respectively. The authors commented that these results compared favorably to the results of the 2NN study. For comparison, the median CD4+ count in the efavirenz and nevirapine (bid) arms of the 2NN study were 190 cells/ μ L and 180 cells/ μ L, respectively, and the average CD4+ count rises at 48 weeks were 160 and 160 cells/ μ L, respectively (van Leth et al, 10th CROI, 2003).

Thailand

Sungkanuparph and colleagues presented the results of an analysis of 159 patients starting antiretroviral therapy with CD4+ counts below 50 cells/ μ L in a clinical cohort (Abstract 587). The median baseline CD4+ count was 22 cells/ μ L, and the median baseline HIV-1 RNA was 260,000 copies/mL. There were 14 patients who discontinued antiretroviral therapy due to adverse events, 5 who were lost to follow-up, and 2 who died. Of those remaining on antiretroviral therapy, the median CD4+ count rise at 1 year was 201 cells/ μ L, and the percent with HIV RNA levels below 400 copies/mL (and <50 copies/mL) was 91% (and 79%).

Conclusions

In 2004 the CROI maintained its position as the preeminent research conference of the year, presenting state-of-the-art information, including that concerning advances in antiretroviral therapy. New drugs on the horizon, updated knowledge on how to better use available drugs, the implications of viral resistance, and the internationalization of antiretroviral therapeutics presented a picture of cautious hope for continued improvements in care for HIV-infected individuals worldwide.

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Conference Abstracts cited in text can be found at www.retroconference.org.

Additional Suggested Readings

Cahn P, Lange J, Cassetti I. Anti HIV-1 activity of SPD754 a new NRTI: results of a 10 day monotherapy study in treatment naive HIV patients. [Abstract LB15.] 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment. July 13-16, 2003; Paris, France.

Derdeyn CA, Decker JM, Bibollet-Ruche F, et al. Selective Heterosexual Transmission of Envelope-constrained, Neutralization-sensitive HIV-1 [Abstract 127]. 10th Conference on Retrovirus and Opportunistic Infections. February 10-14, 2003; Boston, Mass.

Van Leth F, Hassink E, Phanuphak P, et al. Results of the 2NN study: A randomized comparative trial of first-line antiretroviral therapy with regimens containing either nevirapine alone, efavirenz alone or both drugs combined, together with stavudine and lamivudine. [Abstract 176.] 10th Conference on Retroviruses and Opportunistic Infections. February 10-14, 2003; Boston, Mass.