

# Antiretroviral Therapy Update

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*As in prior years, antiretroviral therapy represented a substantial proportion of the abstract presentations at the conference. The information presented extended a number of themes from last year's conference, including an update on the status of new antiretroviral agents; the efficacy of various regimens as part of initial therapy or alternative therapy in the setting of virologic failure; strategic approaches to antiretroviral management; the increasingly recognized importance of maintaining adequate drug levels for successful virologic suppression; the relevance of cellular and tissue compartments as viral reservoirs; and the relevance of drug resistance, including the description of new genotypic patterns and the utility of resistance testing in clinical management. This review will highlight the specific presentations that contributed to these themes.*

## New Investigational Drugs

Data were presented on investigational compounds that continue to progress through more advanced stages of clinical testing or that show promising preclinical antiviral and pharmacokinetic properties.

### Reverse Transcriptase Inhibitors

**DAPD.** Richman and colleagues reported the results of a nonrandomized, Phase I/II trial of the dioxolane guanosine analogue, DAPD (Abstract 668). This nucleoside reverse transcriptase inhibitor (nRTI) is rapidly absorbed and then deaminated by adenosine deaminase to dioxolane guanine (DXG), a compound with a half-life of approximately 7 hours (Abstract 103). DXG has previously been shown to retain in vitro activity against HIV-1 isolates containing lamivudine- and zidovudine-associated resistance mutations. The DAPD dosages, ranging from 25 mg to 300 mg twice a day, re-

sulted in dose-related maximum median plasma HIV-1 RNA reductions from 0.45 log<sub>10</sub> to 1.5 log<sub>10</sub> copies/mL in the 2-week study. The drug was well tolerated, and no reverse transcriptase genotypic changes were seen.

**Foscarnet Analogues.** Hostetler and colleagues reported on the in vitro antiretroviral effects of alkylglycerol analogues of phosphonoformate (foscarnet) (Abstract 666). The 2 lead compounds in this group, methyl batyl-PFA (MB-PFA) and ethyl batyl-PFA (EB-PFA), exhibit substantially lower 50% inhibitory concentration (IC<sub>50</sub>) values (0.28 μM and 0.39 μM, respectively) than the parent compound PFA (16.3 μM), and encouragingly, retain activity against HIV-1 strains containing key reverse transcriptase drug resistance mutations including M184V, L74V, Q151M, K103N, and assorted zidovudine resistance-associated mutations. Moreover, synergy was noted when these compounds were combined with zidovudine. Addressing 2 major deficiencies of the parent compound, the cytotoxicity indices of these analogues were lower than with PFA (on the order of 10-fold) and in rodent models exhibit good oral bioavailability. Hammond and colleagues (Abstract 736) evaluated mutations arising in vitro by serial passage of HIV-1 isolates in MT-2 cells exposed to MB-PFA and EB-PFA. The observed mutations, W88G, M164I, and S117T, were each coselected with the L214F and resulted in phenotypic resistance. Notably, the L214F mutation was not selected by free phosphonoformic acid. The frequent occurrence of the L214F mutation as a natural polymorphism is relevant to these observations.

**FLT.** Abstract 472 demonstrated the in vitro activity of the thymidine analogue 3'-fluoro-3'-deoxythymidine (FLT) in peripheral blood mononuclear cells (PBMCs) against a variety of drug resistant strains. These include the Q151M

mutation alone as well as the Q151M complex, combinations of zidovudine-resistant isolates (with or without M184V) and the 69 insertion variants 69EA and 69SA. These various drug-resistant isolates demonstrated 5- to 100-fold reduced susceptibilities to zidovudine and the IC<sub>50</sub> values of FLT approximated wild-type (0.0075 μM), ranging from 0.0014 μM to 0.0162 μM. This latter IC<sub>50</sub> was associated with an isolate bearing the 67E/69SSA/R211K/T215. The cytotoxicities observed in PBMCs with FLT were comparable to those observed with zidovudine. Data on hepatotoxicity were not provided in this abstract.

**Capravirine.** Initial pharmacokinetic and safety data were presented on capravirine, an investigational nonnucleoside reverse transcriptase inhibitor (NNRTI) that exhibits in vitro activity with a mean EC<sub>50</sub> and EC<sub>90</sub> of 0.5 and 1.53 μg/L, respectively, to laboratory and clinical HIV-1 isolates (Abstract 669). Importantly, antiviral activity is maintained against strains harboring the K103N, Y188C, and P236L mutations. Pharmacokinetic and virologic parameters were measured on days 1, 5, and 10 in antiretroviral-naïve patients with CD4+ cell counts above 50/μL and plasma HIV-1 RNA levels greater than 5000 copies/mL. The oral bioavailability of capravirine was increased with food and its serum half-life was between 1.3 and 1.99 hours with 700 mg twice-daily dosing. Due to its metabolism by the CYP3A4 P450 isozyme, plasma capravirine levels increased by 2-fold when administered in combination with either nelfinavir or indinavir, and there are no significant reciprocal effects (Abstract 83). At day 10, a dose-related mean reduction in plasma HIV-1 RNA levels was observed; a 700 mg twice-daily dosing resulted in a 1-log<sub>10</sub> copies/mL reduction and the highest dose, 2100 mg twice daily, resulted in a 1.7-log<sub>10</sub> copies/mL reduction. No dose-limiting toxicities were noted.

**Emivirine.** There were several presentations of data resulting from the use of the NNRTI emivirine at this year's conference. Raffi and colleagues reported the 24-week follow-up of the double-blind MKC-302 trial that studied the virologic efficacy of emivirine versus placebo combined with stavudine and didanosine (Abstract 514). The median baseline plasma HIV-1 RNA and CD4+ levels of the 123 antiretroviral-naive subjects were 5.0 log<sub>10</sub> copies/mL and 338 cells/μL, respectively. At week 24, 42% of emivirine/stavudine/didanosine recipients (versus 30% in the placebo arm) had 400 or fewer copies/mL and 25% (versus 8% in the placebo arm) had 50 or fewer copies/mL (intent-to-treat [ITT] analysis). Upon stratifying the 24-week responses based on baseline plasma HIV-1 RNA levels, 52% of emivirine/stavudine/didanosine recipients versus 33% of the dual nRTI subjects with initial plasma viral RNA levels at or below 100,000 copies/mL had less than 400 copies/mL. Thirty-two percent and 27% with baseline levels at or below 300,000 copies/mL reached levels below this level of detection. Failure due to intolerance was more common among those receiving emivirine (15% versus 2%), especially in the first month of therapy.

These results from the MKC-302 trial were pooled with the MKC-301 and MKC-202 trials to obtain a collective 24-week antiviral efficacy, safety, and tolerability analysis for emivirine/dual nRTI regimens (Abstract 671). MKC-301 was a double-blind trial employing emivirine/stavudine/lamivudine and MKC-202 was an open-label trial studying emivirine at 500 mg or 750 mg twice a day with stavudine/didanosine. The 481 NNRTI- and protease inhibitor-naive subjects had pooled baseline median plasma viral loads between 4.55 and 4.66 log<sub>10</sub> copies/mL and median CD4+ cell counts between 340/μL and 390/μL. At week 24, 54% versus 32% and 42% versus 12% of pooled emivirine versus placebo recipients lowered plasma HIV-1 RNA levels to at or below 400 and 50 copies/mL, respectively (ITT analysis). Permanent discontinuation of emivirine due to adverse effects—most commonly, nausea, headache, diarrhea, dizziness, rash, and transaminitis—occurred in 10% of cases versus 1% in the placebo arm. Genotypic

analysis of viral isolates from patients experiencing virologic failure in the emivirine-containing regimens revealed nRTI- and NNRTI-associated resistance mutations in 4% and 15% of cases, respectively, most notably the M184V and K103N mutations.

In a more detailed analysis of emivirine resistance patterns in the 57% of patients failing 24 weeks of emivirine/dual nRTI therapy in the MKC-303 trial, Borroto-Esoda and colleagues also found that K103N was the predominant NNRTI mutation (in 38% of patients in whom therapy was failing) (Abstract 751). Other observed reverse transcriptase gene mutations included K101E, V108I, V179I, Y181C, Y188H or C, and G190A. Isolates that contained the K103N or K101E mutations were cross-resistant to nevirapine, delavirdine, and efavirenz. In addition, the Y181C mutation was more likely to develop in patients in whom emivirine/nelfinavir/stavudine/didanosine was failing (31.25%) than in patients in whom emivirine/nelfinavir/stavudine/lamivudine was failing (3.7%).

**Other Investigational NNRTIs.** Other preliminary pharmacokinetic and virologic data were presented on 3 additional investigational NNRTIs: GW420867X, DPC 083, DPC 961, and (+)-calanolide A. In the NNR20001 study, the quinoxaline derivative, GW420867X, was administered as monotherapy for 7 days at 3 different dosages (50 mg to 200 mg qd), then combined with zidovudine/lamivudine for 21 days (Abstract 507). Virologic responses were seen in all dosage groups with a median -2 to -2.5 log<sub>10</sub> copies/mL change in plasma HIV-1 RNA levels. Viral suppression to less than 400 copies/mL was demonstrated even in 2 patients with baseline M184V and K103R reverse transcriptase genotypes. The development of GW4208678X, however, has been stopped.

In 2 posters, the pharmacokinetic characteristics of 2 NNRTIs, DPC 961 and DPC 083, were presented (Abstracts 99 and 102). These compounds have previously been shown to have promising in vitro activity, most notably against HIV-1 strains with K103N and L100I single mutations or double mutations (eg, K103N and Y181C, V108I, or P225H) associated with nevirapine, delavirdine, and

efavirenz treatment failure. Administered to healthy volunteers in once-daily doses, DPC 083 and DPC 961 were associated with prolonged terminal half-lives (>140 hours and 49 + 22 hours, respectively) and attained protein-binding-adjusted concentrations above the EC<sub>90</sub> of wild type and the NNRTI-resistant mutant strains noted above. The development of DPC 961 is currently on hold.

In a Phase IB study of (+)-calanolide A, a modest -0.81 log<sub>10</sub> copies/mL, dose-dependent antiviral effect in plasma HIV RNA (highest with 600 mg bid) was demonstrated (Abstract 508). No reverse transcriptase genotypic changes were noted over the 2-week study period.

### Investigational Protease Inhibitors

Therapeutic goals for new HIV-1 protease inhibitors echo those that have prompted the development of "second generation" reverse transcriptase inhibitors; namely, to produce drugs that retain antiviral activity against viral isolates resistant to the current generation of agents. Preliminary clinical data on 2 promising second generation protease inhibitors, tipranavir and BMS-232632, were presented at the conference.

**Tipranavir.** Wang and colleagues presented data from tipranavir protocol 0015, a Phase II safety and efficacy study of tipranavir alone or in combination with low dose ritonavir (200 mg bid), administered as a pharmacokinetic enhancer of tipranavir rather than as a second protease inhibitor (Abstract 673). The 31 enrolled treatment-naive patients were randomized to 3 open-label groups that received tipranavir 1200 mg twice daily (group 1), tipranavir 300 mg twice daily/ritonavir 200 mg twice daily (group 2), or tipranavir 1200 mg twice daily/ritonavir 200 mg twice daily (group 3). In a pharmacokinetic subanalysis, all 3 regimens achieved median tipranavir trough concentrations that exceeded the target level of 0.5 to 1.0 μM that was based on the results of previous in vitro susceptibility testing. However, the area under the curve (AUC) for tipranavir/ritonavir in groups 2 and 3 was approximately 5-fold and 14-fold greater than with tipranavir alone. Because tipranavir is an inducer of CYP3A4, an expected 5-fold reduction in

ritonavir systemic exposure was expected, again emphasizing the role of ritonavir as a pharmacokinetic enhancer rather than as an antiviral drug in this combination. Significantly enhanced short-term virologic and immunologic efficacy were demonstrated with the tipranavir/ritonavir arms relative to tipranavir alone. The 15-day plasma HIV-1 RNA reductions in groups 1, 2, and 3 were 0.7 log<sub>10</sub>, 1.5 log<sub>10</sub>, and 1.6 log<sub>10</sub> copies/mL, respectively. CD4+ increases over baseline were 10 to 25 cells/μL in group 1 and 60 to 90 cells/μL in groups 2 and 3. The most common adverse effects were gastrointestinal (GI)-related: loose stools or diarrhea (52%), nausea (23%), and vomiting (13%).

**BMS-232632.** The results of the AI 424-007 Phase II trial, which compared the safety and virologic efficacy of BMS-232632, were reported by Sanne and colleagues (Abstract 672). BMS-232632 is an aspartyl protease inhibitor with an EC<sub>50</sub> of 2 to 5 nM. Oral bioavailability is increased by 35% and 79% with high-fat and light meals, respectively; a 400 mg once-daily dose achieves mean steady-state concentrations above the protein binding-adjusted IC<sub>90</sub> (Abstract 504). In the AI 424-007 Trial, 91 antiretroviral-naive patients with a median plasma HIV-1 RNA level of 4.80 log<sub>10</sub> copies/mL and CD4+ cell count of 390/μL received BMS-232632 (200 or 400 mg qd) monotherapy for 2 weeks, which was then combined with didanosine and stavudine. In an on-treatment (OT) analysis, there was an approximate median decline of 1.5 log<sub>10</sub> copies/mL in the plasma HIV-1 RNA level after the 2-week monotherapy period and a decline of 2.0 to 2.5 log<sub>10</sub> copies/mL after 16 weeks. At week 12, CD4+ cells increased by 80/μL to 120/μL. Reversible indirect hyperbilirubinemia was the only dose-limiting effect, typically occurring in the first 60 days of treatment.

### Options for Initial Therapy: Clinical Trials in Antiretroviral-Naive Patients

The clinical trials in antiretroviral-naive subjects reported at the conference ad-

ressed the following central issues concerning the initiation of antiretroviral therapy: (1) the long-term durability of virologic and immunologic efficacy of previously reported potent combination regimens; (2) the relative antiretroviral efficacy of “protease-sparing” regimens compared with “standard” protease inhibitor/dual nRTI therapy; (3) the influence of the timing of therapy initiation on subsequent efficacy; (4) other factors that might predict virologic response in therapy-naive individuals; (5) the efficacy of streamlined dosing schedules that might promote better drug adherence; and (6) virologic and immunologic effects of therapy initiated during primary HIV-1 infection. These trials are summarized in Table 1.

### Extended Follow-up of Previously Reported Trials

**DMP-266-003-Cohort IV Study.** The long-term efficacy and safety of an indinavir/efavirenz combination regimen in 59 protease inhibitor- and NNRTI-naive subjects from Study 003-Cohort IV were reported by Riddler and colleagues (Abstract 513). The original multicenter, randomized, double-blind trial compared indinavir dosed at either 800 mg or 1000 mg 3 times a day with indinavir (at the same dosing schedules) and efavirenz 200 mg once a day. Extended data from the indinavir/efavirenz study arm (Cohort IV) demonstrated that at 132 weeks of study, 66% of subjects maintained plasma HIV-1 RNA levels of less than 400 copies/mL from a baseline mean of 5.09 log<sub>10</sub> copies/mL in an ITT analysis where nonadherence equals failure. Concurrently, there was a mean increase of 350 CD4+ cells/μL in this group. Adverse events led to treatment discontinuation in approximately 5% of subjects; diarrhea and nausea were the most common toxicities.

**M97-720 Study.** Gulick and the M97-720 Study Group reported the 72-week efficacy data from the use of ABT-378/ritonavir with stavudine/lamivudine in 100 antiretroviral-naive subjects (Abstract 515). Study participants initially received ABT-378/ritonavir in one of 3 twice-daily dosing levels: 200/100 mg,

400/100 mg, or 400/200 mg. At week 48, all patients received the 400/100 mg twice-daily dosing. The dual nRTI portion was administered either after 3 weeks of ABT-378/ritonavir (group 1, an evaluation of preliminary ABT-378/ritonavir safety and efficacy) or at study entry (group 2). Pooled baseline median CD4+ cell and plasma HIV-1 RNA levels were approximately 400/μL and 4.8 log<sub>10</sub> copies/mL, respectively. Overall, in an ITT analysis where missing values equal failure (M=F), 82% of patients had reductions in plasma HIV-1 RNA levels to less than 400 copies/mL and 80% to less than 50 copies/mL. When stratified by baseline plasma viremia above or below 5 log<sub>10</sub> copies/mL, the virologic response rates (attaining <400 copies/mL) were similar subsequent to week 20.

**CNA2006 Study.** Rizzardì and colleagues presented the long-term (72-week) results of CNA2006, an open-label, non-randomized observational study employing a combination of amprenavir and abacavir (Abstract 336). Of the 41 individuals enrolled in this trial, 32 were followed to 72 weeks (see Table 1). At this time point, 68% of subjects had plasma HIV-1 RNA levels below 50 copies/mL and about 40% below 5 copies/mL from a mean level of 4.42 log<sub>10</sub> copies/mL at study entry (ITT analysis). In an OT analysis, viral loads were reduced to below these 2 levels in 88% and about 50% of subjects, respectively. Substantial increases in total CD4+ cells and the naive CD4+ cell subset were also noted, with mean increases from baseline of approximately 215 and 80 cells/μL, respectively. Over the study period, lymph node CD4+/CD8+ ratios normalized and HIV-1-specific lymphocyte proliferative responses were partially restored.

### Protease-Sparing Regimens

Several studies presented at the conference compared the relative virologic and immunological efficacy of more standard single protease inhibitor-based combination therapy with NNRTI- and triple nRTI-based combination therapy.

The relative efficacy of an initial NNRTI- versus protease inhibitor-based regimen was examined by Podzamczar and colleagues, who presented the 6-

month results of the COMBINE Study (Abstract 510). This was a randomized multicenter trial that compared the marker efficacy of fixed formulation zidovudine/lamivudine combined with either nelfinavir (1250 mg bid) or nevirapine. A total of 143 treatment-naive subjects with median baseline CD4+ cell and plasma HIV-1 RNA levels of 364/ $\mu\text{L}$  and 4.8  $\log_{10}$  copies/mL were enrolled. Although immunologic responses were comparable in a 6-month ITT analysis, there was a significantly greater number of subjects in the nevirapine arm in whom plasma HIV-1 RNA levels were reduced to less than 20 copies/mL, even in those who had baseline plasma viral loads of more than 5  $\log_{10}$  copies/mL.

The relative marker efficacy of a triple nRTI regimen versus a standard protease inhibitor-based regimen was investigated by Demarest and colleagues in a total of 527 antiretroviral-naive patients in the randomized, double-blind parallel group, multicenter CNA3005 trial (Abstract 331). Subjects were randomized to zidovudine/lamivudine combined with abacavir or indinavir. At 48 weeks, without a stratification by baseline plasma viral load, and consistent with the 24-week results reported at the 1999 Retrovirus Conference, there continued to be comparable virologic responses between the 2 study arms. This was reflected by 51% of subjects in both groups attaining plasma HIV-1 RNA levels at or below 400 copies/mL by ITT analysis. However, in the subset of patients with baseline plasma HIV-1 RNA levels above 5  $\log_{10}$  copies/mL, a greater percentage of indinavir recipients reduced these levels to at or below 50 copies/mL. Comparable immune restoration was noted in both groups as reflected by similar median CD4+ cell increases (approximately 140 cells/ $\mu\text{L}$ ), increased thymic production of T cells (as measured by T-cell receptor rearrangement excision circles), and CD4+ VB CDR3 T-cell receptor repertoires. In individuals in whom therapy was failing, viral isolates were most commonly either genotypically wild-type (14/44 patients) or contained the M184V reverse transcriptase mutation (Abstract 750).

Addressing the relative efficacy of protease-sparing initial regimens in a meta-analysis, Bartlett and colleagues

found that the antiviral effects of protease inhibitor, NNRTI, and triple nRTI regimens in antiretroviral-naive patients were comparable (Abstract 519). The 18 studies included in this analysis employed 24 different triple drug combinations that were administered for at least 24 weeks. Virologic efficacy was based on the ability to obtain plasma HIV-1 RNA levels below 50 copies/mL by the Roche Amplicor assay using an ITT analysis at week 24. The median baseline plasma HIV-1 RNA and CD4+ cell count levels were 4.69  $\log_{10}$  copies/mL and 376/ $\mu\text{L}$ , respectively. Of the patients receiving protease inhibitor-, NNRTI-, or triple nRTI-based regimens, an estimated 63% or 49%, 65% or 51%, and 67% or 49% had plasma HIV-1 RNA declines to less than 400 or 50 copies/mL, respectively. There was a small but statistically significant virologic efficacy advantage with the NNRTI-based regimens, mostly attributable to the results of the more recently reported efavirenz-containing trials. The collective mean CD4+ cell increase was 158/ $\mu\text{L}$  at 48 weeks. Examining the factors that were associated with viral suppression, subjects with a higher baseline plasma HIV-1 RNA level (RR=-0.49, P=0.01) or higher pill burden (RR=-0.61, P=0.0056) were less likely to suppress their viral load to <50 copies/mL.

### Timing of Initial Antiretroviral Therapy

Several presentations at this year's conference suggested that, in general, early initiation of antiretroviral therapy may be more effective for viral suppression.

To address the issue of timing of initiation, Meibohm and the Crixivan Study Group compared the relative effects of early versus late initiation of indinavir/zidovudine/lamivudine in 4 previously reported trials (Abstract 521). This combination was employed in patient populations ranging from acute infection (Protocol 042) to early (Protocol 060), intermediate (Protocol 035) and advanced (Protocol 039) stages of chronic HIV-1 disease. Reflective of the respective disease stages, the entry criteria in these 4 studies included CD4+ cell counts ranging from 500/ $\mu\text{L}$  and above to 50/ $\mu\text{L}$  and below. Subjects in the acute and early

stage trials were antiretroviral-naive and in the intermediate and advanced stage trials were zidovudine-experienced. Suggestive that earlier treatment may result in more sustained viral suppression and immune restoration, the proportion of individuals attaining plasma HIV-1 RNA diminution to less than 50 copies/mL were 83%, 80%, 67%, and 47%, respectively, and mean CD4+ cell increases over baseline were 212/ $\mu\text{L}$ , 163/ $\mu\text{L}$ , 187/ $\mu\text{L}$ , and 126/ $\mu\text{L}$ , respectively, at 48 weeks (ITT analysis). The duration of suppression was longest in the acute infection and early stage protocols and the time to suppression was more protracted in the advanced stage protocol.

Moore and colleagues studied 680 patients from the Johns Hopkins HIV Cohort who were treated for at least 120 days (median, 390 days) with a regimen containing a protease inhibitor and at least 2 other drugs (Abstract 522). Stratification by baseline CD4+ cell counts of above 350/ $\mu\text{L}$ , 200/ $\mu\text{L}$  to 350/ $\mu\text{L}$ , and below 200/ $\mu\text{L}$ , showed that 81%, 74%, and 65% of subjects achieved plasma HIV-1 RNA levels of less than 400 copies/mL and that 43%, 34%, and 28% had durable virologic responses in these ranges, respectively. In a multivariate logistic regression analysis for factors that were associated with achieving viral suppression, a baseline CD4+ cell count above 350/ $\mu\text{L}$  was a significant factor (relative odds [RO], 1.9; P=0.03). A baseline plasma HIV-1 RNA of more than 5  $\log_{10}$  copies/mL (RO, 0.69; P=0.04), intravenous drug abuse (RO, 0.68; P=0.04), and missing more than 3 doses (RO, 0.53; P=0.02) were negatively correlated with attaining plasma viral suppression.

In a third study supporting early initiation, McMahon and the Merck 060/ICC 004 Study team presented the 48-week results of this trial examining the efficacy and tolerability of indinavir/zidovudine/lamivudine administered to treatment-naive subjects with early HIV-1 disease (CD4+ cell counts >500/ $\mu\text{L}$ ) (Abstract 511). Mean baseline plasma HIV-1 RNA and CD4+ cell levels were 3.92  $\log_{10}$  copies/mL and 597/ $\mu\text{L}$ , respectively. The proportion of patients achieving plasma HIV-1 RNA reductions to below 400 copies/mL was 82% and to below 50 copies/mL was 79% (ITT analysis). Of 199 enrolled patients, 15 had therapy discon-

tinued due to adverse effects.

A poster presented by Tebas and colleagues tempered the conclusion drawn by the above studies that early therapeutic antiretroviral intervention may be more advantageous (Abstract 523). The authors studied the issue of when to initiate therapy by Markov modeling and decision analysis in order to understand the potential long-term implications of early therapy. The model examined the expected virologic outcomes of immediate therapy versus progressive initiation in a population of 10,000 HIV-seropositive patients over a 10-year period. Stated baseline assumptions of the model, including efficacy (plasma HIV-1 RNA levels <50 copies/mL at 48 weeks) and durability of an initial regimen, and the likelihood of successful salvage therapy, were based on data obtained from several previously reported clinical trials. Potential immunologic benefits of early intervention were not factored into the model. Notable conclusions were that progressive delayed initiation of therapy would result in improved virologic outcomes and less prevalent multidrug-resistant virus in the long term compared with early intervention; that the overall drug-related cost would be less with progressive initiation; and that immediate initiation should be at least 15% to 20% better than progressive initiation in order to support the use of immediate therapy.

### **Other Potential Factors Predicting Virologic Response in Therapy-Naive Subjects**

A group of conference studies sought to better define other factors that might predict a durable response in an initial antiretroviral regimen. In selected studies, these factors included specific baseline marker levels and protease inhibitor trough levels.

Acosta and colleagues presented data from ACTG 878, the pharmacokinetic substudy of ACTG 343 (Abstract 455). The ACTG 343 trial assessed the efficacy of an induction-maintenance strategy using indinavir/zidovudine/lamivudine induction in protease inhibitor-naive subjects, then randomizing them to one of 3 maintenance regimens: indinavir/zidovudine/lamivudine, indinavir monotherapy, or zidovudine/lamivudine. ACTG 878 exam-

ined the pharmacologic factors associated with viral suppression in the 3-drug maintenance cohort of 179 patients. In these individuals, modeled indinavir trough levels were statistically different between those with plasma HIV-1 RNA levels below and those with levels above 200 copies/mL at week 24 ( $P=0.006$ ). There was a significant association ( $RR=0.34$ ,  $P<0.05$ ) between the week 4 indinavir trough level and the  $\log_{10}$  change in plasma HIV-1 RNA at week 4. The 90% response rate corresponded to an indinavir trough level of 110 ng/mL.

Pollard and the 1090 team investigated factors that may have influenced response to nevirapine/zidovudine/lamivudine (Abstract 517). This virologic substudy of a 2256-subject trial consisted of 200 antiretroviral-naive individuals with respective baseline mean plasma HIV-1 RNA and CD4+ cell levels of 5.15  $\log_{10}$  copies/mL and 97/ $\mu$ L. Patients were randomized to zidovudine/lamivudine combined with nevirapine or placebo. In a logistic regression analysis, a sustained virologic response (ie, plasma HIV-1 RNA level of <50 copies/mL at week 48) was associated with the nevirapine study arm ( $P=0.0001$ ) and a higher baseline CD4+ cell count ( $P=0.0012$ ), but not with a lower baseline plasma viral load ( $P=0.8044$ ).

Rizzardi and colleagues investigated potential virologic and immunologic factors that predict the length of time required for full suppression of circulating virus, as defined by a plasma HIV-1 RNA level below 50 copies/mL (Abstract 520). Antiretroviral-naive patients ( $n=118$ ) enrolled in several different clinical trials (eg, CNA2006, the AVIB study, NUC2019, the ADAM study, CNA3005) were assessed for baseline and post-treatment virologic and immunologic parameters in both blood and lymph nodes. The baseline plasma HIV-1 RNA level was the best predictor of the time to plasma viral suppression ( $RR=0.48$ ,  $P<0.0001$ ), followed by the baseline numbers of virus-expressing lymph node cells ( $RR=0.45$ ,  $P=0.001$ ) and follicular dendritic cell-associated HIV-1 RNA levels ( $RR=0.31$ ,  $P=0.032$ ). A similar analysis was conducted for the length of time required to suppress plasma HIV-1 RNA levels to below 5 copies/mL. To this lower level of detection, none of the baseline

marker parameters were significantly correlated. Both the plasma and lymph node viral load had comparable abilities to estimate the time required to reach a plasma HIV-1 RNA below 50 copies/mL: 73 and 83 days, respectively.

A possible influence of gender in subsequent virologic response was investigated in the START I and II trials (Abstract 529). The purpose of these parallel, 48-week, randomized trials was to examine whether gender differences in efficacy and toxicity of thymidine analogue-containing, 3-drug regimens could be detected. A total of 78 women and 331 men, all antiretroviral-naive, were randomized to indinavir combined with zidovudine/lamivudine or stavudine/lamivudine (START I) or indinavir combined with zidovudine/lamivudine or stavudine/didanosine (START II). No significant differences in marker responses or toxicities were noted between gender or treatment arms.

### **Streamlined Dosing of Initial Treatment Regimens**

Molina and colleagues reported the preliminary safety and efficacy data from the ANRS 091 trial, a pilot study of a once-a-day combination regimen of emtricitabine (FTC)/didanosine/efavirenz (Abstract 518). The 40 enrolled patients had a median CD4+ cell count of 373/ $\mu$ L and plasma HIV-1 RNA level of 4.77  $\log_{10}$  copies/mL. After 24 weeks, 98% and 93% had plasma viral loads to below 400 and to below 50 copies/mL, respectively. Concurrently, the mean CD4+ cell increase was 151/ $\mu$ L. The major adverse effects were neurologic (ie, dizziness, sleep disturbance, depression, headache, asthenia), gastrointestinal (ie, diarrhea, abdominal pain), and rash.

### **Primary HIV-1 Infection Therapy**

Cooper and colleagues reported the results of the QUEST Study, a multicenter trial investigating amprenavir/abacavir/zidovudine/lamivudine for the treatment of primary HIV-1 infection (Abstract 552). At 28 weeks, the mean baseline total CD4+ cell count increased by 249/ $\mu$ L; the CD45RA+ subpopulation increased by 87/ $\mu$ L, and CD4+CD45 RO+ by 160/ $\mu$ L. The total mean number of CD8+ cells de-

Table 1. Trials in Antiretroviral-Naive Patients

Study Name (Abstract Number)	Regimen/Study Arm	Number of Patients	Weeks of Therapy	Baseline Plasma HIV-1 RNA copies/mL
<b>COMBINE Study (510)</b>	Nelfinavir/Zidovudine/Lamivudine	143 (total of 2 study arms)	24	4.81 log <sub>10</sub> (median)
	Nevirapine/Zidovudine/Lamivudine		24	4.77 log <sub>10</sub> (median)
<b>CNA2006 Study (336)</b>	Amprenavir/Abacavir	41 (32 completed the 72 week follow-up)	72	4.42 log <sub>10</sub> (mean)
<b>DMP-003 Cohort IV (513)</b>	Indinavir/Efavirenz	59	132	5.09 log <sub>10</sub> (mean)
<b>CNA3005 Trial (331)</b>	Abacavir/Zidovudine/Lamivudine	262	48	4.88 log <sub>10</sub> (overall median)
	Indinavir/Zidovudine/Lamivudine	265	48	
<b>M97-720 Study (515)</b>	ABT-378/Ritonavir/Stavudine/Lamivudine	100	72	4.8 log <sub>10</sub> (overall median)
<b>START I Trial (529)</b>	Zidovudine/Lamivudine/Indinavir	103	48	4.38 log <sub>10</sub> (overall mean for females)
	Stavudine/Lamivudine/Indinavir	101		4.56 log <sub>10</sub> (overall mean for males)
<b>START II Trial (529)</b>	Zidovudine/Lamivudine/Indinavir	103	48	
	Stavudine/Didanosine/Indinavir	102		
<b>Merck 060/ICC 004 Study (511)</b>	Zidovudine/Lamivudine/Indinavir	199	48	3.92 log <sub>10</sub> (mean)
<b>ANRS 091 Trial (518)</b>	Emtricitabine (FTC)/Didanosine/Efavirenz	40	24	4.77 log <sub>10</sub> (median)
<b>MKC-302 Trial (514)</b>	Emivirine/Stavudine/Didanosine	24	123	5.0 log <sub>10</sub> (median)

ITT indicates intent-to-treat analysis; OT indicates on-treatment analysis. NA=F indicates that nonadherence equals failure; M=F indicates that missing values equal failure.

Baseline CD4+ cells/ $\mu$ L	Plasma HIV-1 RNA Change	CD4+ Cell/ $\mu$ L Change	Comments
347 (mean)	60% <200 copies/mL 33% <20 copies/mL	498 (+151)	ITT analysis of virologic efficacy. The proportion of patients with >5 log <sub>10</sub> copies/mL baseline plasma HIV-1 RNA level reaching <200 or 20 copies/mL after 6 months was also significantly greater in the nevirapine arm (P=0.03 and 0.01, respectively, in both ITT and observed analyses).
369 (mean)	74% <200 copies/mL (P=0.06) 58% <20 copies/mL	527 (+158) (P=0.63)	
756 (mean)	68% <50 copies/mL Approximately 40% <5 copies/mL (ITT, NA=F)	970 (Naive CD4+ cells increased by about 80/ $\mu$ L)	The most common adverse effects were nausea, diarrhea, headache, oral/perioral paresthesias, and fatigue. Increased cholesterol levels were noted.
283 (mean)	66% <400 copies/mL (ITT, NA=F) 91% <400 copies/mL (observed)	350 (mean)	The efavirenz dose was increased to 600 mg qd after at least 36 weeks. Patients randomized to the 2 indinavir monotherapy arms were allowed to add efavirenz/stavudine at week 12. In an ITT analysis at week 132, 39% of these patients had plasma HIV-1 RNA levels <400 copies/mL (P 0.05).
360 (overall median)	51% 400 copies/mL (ITT)  51% 400 copies/mL (ITT)	+149 (median, ITT)  +142 (median, ITT)	In patients with baseline plasma HIV-1 RNA >5 log <sub>10</sub> copies/mL, a greater percentage of indinavir recipients achieved levels <50 copies/mL at 48 weeks.
399 (overall median)	82% <400 copies/mL 80% <50 copies/mL (ITT, M=F) 98% <400 copies/mL 96% <50 copies/mL (OT)	+304 cells/ $\mu$ L (Group 1)  +240 cells/ $\mu$ L (Group 2) (mean)	Adherence rate was 98% through 72 weeks. The most common adverse effects were diarrhea and nausea. Elevated cholesterol and triglyceride levels were seen in 12% to 15% of subjects.
415 (overall mean for females)	40% of females on zidovudine- and stavudine-containing arms with <50 copies/mL	+190 (zidovudine) and +247 (stavudine) increases in females	No significant differences in marker responses or adverse events occurred between gender or treatment groups.
432 (overall mean for males)	Respective 41% and 46% of males on zidovudine- and stavudine-containing arms with <50 copies/mL (ITT)	+183 (zidovudine) and +234 (stavudine) increases in males	
597 (mean)	Approximately 82% <400 copies/mL and 79% <50 copies/mL (ITT)  Approximately 92% <400 copies/mL and 90% <50 copies/mL (observed)	Approximately +150 (mean; both ITT and observed)	Nephrolithiasis was reported in 16 patients.
373 (median)	98% <400 copies/mL and 93% <50 copies/mL (ITT)	+151 (mean)	Of patients with baseline plasma HIV-1 RNA levels $\leq$ 5 log <sub>10</sub> copies/mL, 8/8 had levels < 50 copies/mL at week 24. One patient discontinued therapy due to adverse effects.
338 (median)	42% 400 copies/mL 25% 50 copies/mL (ITT)		Rash (7%) and gastrointestinal (7%) adverse effects were the most common causes for emivirine discontinuation.

creased by 353/ $\mu$ L and mean CD8+CD38+ cells decreased by 775/ $\mu$ L. At week 36, 87% and 58% of subjects had plasma HIV-1 RNA levels below 50 copies/mL and below 5 copies/mL, respectively (ITT analysis). High baseline cell-associated messenger RNA (mRNA) was highly associated with a lower likelihood of subsequent virologic suppression (plasma HIV-1 RNA <50 copies/mL) and was the only independently predictive factor in a multivariate analysis (RR=0.21, P=0.02). Adverse effects associated with the study regimen, mainly gastrointestinal, led to changing 1 drug component of the regimen for 21 subjects.

Several studies characterized the antiviral effect of potent antiretroviral regimens in non-plasma body compartments in primary HIV-1 infection. Pilchnet and colleagues found, in general, uniform virologic efficacy of didanosine/stavudine/nevirapine/hydroxyurea in the concurrently-measured plasma, cerebrospinal fluid (CSF), saliva, semen, and cervicovaginal lavage fluid of 8 patients with primary infection (Abstract 556). At baseline, mean HIV-1 RNA levels in these 5 body fluids were 5.4 log<sub>10</sub>, 3.1 log<sub>10</sub>, 3.3 log<sub>10</sub>, 4.3 log<sub>10</sub>, and 3.7 log<sub>10</sub> copies/mL, respectively. After initiation of therapy, 6 of 9 patients had plasma HIV-1 RNA levels below 50 copies/mL at week 24; 6 of 7 had seminal RNA levels below 400 copies/mL; and 9 of 9 had HIV RNA levels below detection in saliva and CSF. The treatment regimen, however, was associated with a high incidence of treatment-limiting peripheral neuropathy (Abstract 559).

Yerly and colleagues and others demonstrated that despite sustained suppression of plasma HIV-1 RNA levels with potent antiretroviral regimens initiated in primary HIV-1 infection, there is a persistent, slowly decaying or stable low-level cell-associated HIV-1 RNA and DNA load in PBMCs and lymph node tissue (Abstracts 210, 557, and 561).

## Pharmacology

Numerous descriptions of pharmacokinetic interactions with antiretrovirals were reported and are selectively summarized below and in Table 2.

### Three-Way Pharmacokinetic Interactions in NNRTI/Dual Protease Inhibitor Regimens

Blaschke and colleagues found that a significantly lower saquinavir AUC results from a staggered pre-dosing of saquinavir with either ritonavir or nelfinavir, compared with simultaneous administration of ritonavir/saquinavir or nelfinavir/saquinavir (Abstract 76). The pharmacokinetic profile of amprenavir/ritonavir was described in HIV-seronegative volunteers in Abstract 77. Ritonavir increased the amprenavir steady state C<sub>min</sub> by 1325%, probably by its inhibitory effect on CYP3A4. No significant reciprocal effect of amprenavir on ritonavir pharmacokinetics was seen. In ritonavir/amprenavir/efavirenz, dual protease inhibitor/NNRTI regimens, the inhibition of amprenavir metabolism by ritonavir (200 or 500 mg) can compensate for the amprenavir AUC-lowering effect of efavirenz (Abstract 78). Similarly, the 3-way pharmacokinetic interactions in a ritonavir/saquinavir/efavirenz regimen (400 mg/400 mg/600 mg, respectively) are characterized by the balancing of efavirenz CYP3A4-inducing effects by the inhibiting effects of ritonavir (Abstract 79). In this study, doubling the saquinavir dose from 400 mg to 800 mg caused a reciprocal reduction of ritonavir plasma concentrations by 28% and therefore is not recommended.

### Compartmental Pharmacokinetics and Efficacy of Antiretroviral Drugs

Continued evaluation of the central nervous system (CNS) and genitourinary pharmacokinetic and antiviral efficacy of specific drugs has prompted concerns over latent viral reservoirs and over the potential for a loss of the benefits of combination therapy in body compartments that pose a pharmacologic challenge to 1 or more drugs in a regimen. Selected compartmental pharmacokinetic characteristics of specific drugs are outlined in Table 3.

To better define whether differential virologic responses occur between plasma and CSF, Letendre and colleagues examined 20 antiretroviral-naïve and -experienced subjects who were given at least 3 new drugs at study entry and who

had a decrease in plasma HIV-1 RNA of at least 0.5 log<sub>10</sub> copies/mL (Abstract 305). By measuring HIV-1 RNA levels in matched CSF and plasma every 6 to 12 weeks, the authors found that initiating or changing therapy reduced CSF HIV-1 RNA levels in 82%, but half of these patients only responded in CSF and not in plasma. CSF virologic response was statistically associated with greater antiviral responses in the plasma and higher baseline CD4+ cell counts. Virologic responses in CSF and plasma were associated with a higher CSF RNA level at baseline and the initiation of new agents. Delayed responses after 12 weeks of new therapy were more common in the CSF (84%) than in plasma (34%). In the longer-term follow-up (>12 weeks), the CSF responses were more durable. Of those who initially responded in both compartments, 50% maintained CSF suppression and only 25% maintained suppression in both CSF and plasma.

In ACTG 347, Murphy and colleagues also described cases of discordant virologic responses between plasma and CSF in the presentation of the pharmacokinetic and antiviral characteristics of CSF. The randomized trial compared amprenavir with amprenavir/zidovudine/lamivudine (Abstract 314). The 31 protease inhibitor- and lamivudine-naïve subjects in this substudy had median baseline plasma, CSF HIV-1 RNA, and CD4+ cell levels of 4.67 log<sub>10</sub> copies/mL, 3.44 log<sub>10</sub> copies/mL, and 208/ $\mu$ L, respectively. Of the 17 patients who had matched follow-up plasma and CSF specimens, the proportion with CSF HIV-1 RNA levels below 500 copies/mL was 63% for those who received amprenavir monotherapy and 100% for those who received triple therapy. Three patients had discordant responses in plasma versus CSF: 1 with undetectable levels in CSF but detectable plasma levels, and the other 2 with the converse findings.

## Anitretroviral Trials in Treatment Experienced Subjects

### Trials in Subjects with NNRTI Experience

ACTG 370 (Abstract 525) examined a cohort of subjects on stable dual nRTI

therapies with plasma viral loads above 500 copies/mL and who were protease inhibitor- and NNRTI-naive at entry. Subjects receiving stavudine/lamivudine or didanosine/lamivudine at baseline were randomized to either zidovudine/delavirdine/indinavir (n=31) or zidovudine/lamivudine/indinavir (n=33). Subjects receiving zidovudine/lamivudine were switched to stavudine/delavirdine/indinavir (n=41). The median baseline CD4+ cell count and plasma HIV RNA level were 511/ $\mu$ L and 3.06 log<sub>10</sub> copies/mL. At 48 weeks, 77% (23/30), 48% (19/40) and 39% (13/33) of subjects on zidovudine/delavirdine/indinavir, stavudine/delavirdine/indinavir, and zidovudine/lamivudine/indinavir had plasma viral loads of less than 50 copies/mL, respectively (P=0.005 between zidovudine/delavirdine and zidovudine/lamivudine arms). The mean CD4+ cell count increases across the arms ranged from 120 to 145/ $\mu$ L. Thus, among these NNRTI- and protease inhibitor-naive subjects in whom dual nRTI therapy was failing, switching lamivudine for delavirdine provided RNA suppression superior to the continuation of lamivudine in the new regimen. This trial also followed a parallel cohort of subjects treated with one of the dual nRTI regimens described above and who had baseline HIV RNA levels of less than 500 copies/mL (n=54). This group continued the respective dual nRTI regimens into this trial. At 48-week follow-up, 77% (42/54) had plasma viral load levels that remained less than 500 copies/mL on stable dual nRTI therapy.

ACTG 368 (Abstract 529) evaluated the regimens of indinavir/efavirenz/abacavir (n=140) and indinavir/efavirenz/placebo (n=143) in highly nRTI-experienced subjects with CD4+ cell counts below 250 cells/ $\mu$ L in a blinded, randomized, controlled trial. The median baseline CD4+ cell count and plasma viral load was 133/ $\mu$ L and 4.3 log<sub>10</sub> copies/mL. At 48-week follow-up, 69% and 74% of subjects had plasma viral loads of less than 500 copies/mL in the placebo arm and abacavir arm, respectively (P=0.57). The CD4+ cell count increases in the abacavir and placebo arms were comparable (115/ $\mu$ L and 120/ $\mu$ L, respectively).

Albrecht and colleagues (Abstract 531) presented the 40- to 48-week analysis of ACTG 364. Protease inhibitor- and

NNRTI-naive subjects with heavy nRTI experience were randomized to 1 or 2 new nRTIs plus either nelfinavir (n=66) or efavirenz (n=65) or nelfinavir/efavirenz (n=64). At 40 to 48 weeks of follow-up, the proportions of subjects with plasma HIV RNA levels less than 50 copies/mL were 22%, 44%, and 67% in the nelfinavir, efavirenz, and nelfinavir/efavirenz arms, respectively (3-way P=0.001). The mean CD4+ cell count increase from baseline across the 3 arms was 94/ $\mu$ L. In multivariate analysis, the factors predictive of a viral load of less than 50 copies/mL at 40- to 48-week follow-up included treatment with nelfinavir (OR=0.12) or efavirenz (OR=0.34) (relative to nelfinavir/efavirenz, P<0.001), and a decline in plasma viral load to less 50 copies/mL by week 16 (P<0.001). Analysis of baseline reverse transcriptase genotypes in the 146 of 195 study patients with baseline HIV RNA values of more than 2000 copies/mL demonstrated that high-level zidovudine resistance was present in 43% (n=22), 24% (n=11), and 26% (n=11) of subjects in the nelfinavir, efavirenz, and nelfinavir/efavirenz arms, respectively. Furthermore, among those subjects with high-level zidovudine resistance, viral suppression to less than 50 copies/mL in follow-up was observed in 23% (5/22), 18% (2/11), and 91% (10/11) of those in the nelfinavir, efavirenz, and nelfinavir/efavirenz arms, respectively.

### **Trials in Subjects with NNRTI and Protease Inhibitor Experience**

**Lopinavir/Ritonavir.** This study enrolled 70 subjects in whom the first antiretroviral regimen was failing. The regimen consisted of a protease inhibitor and 2 nRTIs for at least 3 months (Abstract 532). These subjects were nRTI-experienced but naive to at least 1 nRTI and naive to all NNRTIs. Lopinavir (ABT-378) (400 mg bid)/ritonavir (100 or 200 mg bid) was substituted for the “failing” protease inhibitor. On day 15, nevirapine was added and the nRTIs were changed to include at least 1 new nRTI. Prior protease inhibitor experience included indinavir (44%), nelfinavir (36%), saquinavir (13%), ritonavir (6%), and amprenavir (1%). The median baseline CD4+ cell count and HIV RNA were 349/ $\mu$ L and 4.0 log<sub>10</sub> copies/mL.

At 48 weeks of follow-up, 60% (42/70, ITT analysis, M=F) and 76% (41/54, OT analysis) had plasma viral loads of less than 50 copies/mL. The mean CD4+ cell count increase at 48 weeks was 125/ $\mu$ L. At baseline, 32% (18/57) of subjects demonstrated a 4-fold or greater reduced susceptibility to 3 or more protease inhibitors and 19% (11/57) had a 4-fold or greater reduced susceptibility to lopinavir. Of these subjects, 7 of 11 had plasma viral loads of less than 400 copies/mL at 48 weeks. The regimen was generally well tolerated, with 12 of 70 patients withdrawing at or before 48 weeks. Only 3 of the 12 were study-drug associated (nausea [1], rash [1], and diarrhea [1]).

ACTG 373 (Abstract 526) evaluated the efficacy of a regimen of stavudine/lamivudine/nevirapine/indinavir (1000 mg q8h) in 56 nRTI-experienced subjects previously treated with a regimen of amprenavir monotherapy (n=36) or amprenavir and nRTIs (n=20; 18/20 had prior zidovudine/lamivudine experience). Few patients had prior NNRTI experience. The median baseline CD4+ cell count and plasma HIV RNA level was 346/ $\mu$ L and 4.19 log<sub>10</sub> copies/mL, respectively. At baseline 20% of subjects had plasma viral loads of less than 500 copies/mL. At 48 weeks, 59% (ITT analysis) and 78% (OT analysis) had plasma viral loads of less than 500 copies/mL. Subjects previously treated with amprenavir monotherapy had superior RNA responses (P<0.01). Notably, 22 (39%) stopped 1 or more antiretrovirals and 11 (20%) experienced a grade 3 or higher toxicity. At week 48, the median CD4+ cell count gain was 94/ $\mu$ L.

Hammer and colleagues (Abstract LB7) presented 24-week results of ACTG 398. This study evaluated the safety and efficacy of a dual protease inhibitor salvage regimen in subjects experiencing virologic failure with at least 16 weeks of protease inhibitor exposure. All subjects received abacavir/adeфовir/efavirenz/amprenavir with one of the following: saquinavir-sgc, indinavir, nelfinavir, or placebo. Among the 481 subjects enrolled, the baseline HIV RNA and CD4+ cell counts were 4.7 log<sub>10</sub> copies/mL and 202/ $\mu$ L. Of subjects enrolled, 21%, 53%, and 26% had prior exposure to 1, 2, or 3 protease inhibitors, respectively. At 24 weeks greater proportions of subjects in

**Table 2. Pharmacokinetic Interactions with Antiretroviral Drugs**

Drug	Coadministered Drug(s)	Interaction	Abstract No.
<b>Amprenavir</b>	Ritonavir	Ritonavir increases amprenavir AUC by 2.5-fold.	78
<b>Amprenavir</b>	Nelfinavir	Nelfinavir increases amprenavir AUC by 1.6-fold.	78
<b>BMS-232632</b>	Didanosine or stavudine	Simultaneous administration of BMS-232632 with didanosine or stavudine lowers BMS $C_{min}$ by 89% and AUC by 87%. No reciprocal effect noted.	504
<b>BMS-232632</b>	Saquinavir	Saquinavir plasma AUC increased by 5.4 to 7.1-fold.	504
<b>Tipranavir</b>	Nucleoside reverse transcriptase inhibitors	15% mean decrease in stavudine AUC. 46% mean decrease in zidovudine and didanosine AUCs. 37% mean decrease in lamivudine AUC. (Note: the clinical relevance of these changes may not be significant since intracellular concentrations were not affected).	81
<b>Abacavir</b>	Adefovir	No significant PK interactions. Abacavir PK in adefovir regimens were similar to those in indinavir/abacavir regimens, and adefovir PKs are similar to regimens without abacavir or efavirenz.	85
<b>Adefovir</b>	Protease inhibitors	There is a non-clinically significant adefovir AUC increase by 20% caused by saquinavir and food. Indinavir PK is unchanged.	86
<b>Adefovir</b>	Reverse transcriptase inhibitors	Adefovir increases didanosine AUC by 29%, probably by competing for a common active tubular secretion pathway, but this is probably not of clinical significance. Lamivudine, delavirdine, and efavirenz PKs are unchanged.	86
<b>Methadone</b>	Efavirenz or nevirapine	Nevirapine reduces methadone AUC by 46%. Efavirenz reduces methadone AUC by almost 60%. Methadone withdrawal symptoms need to be differentiated from efavirenz CNS toxicity. Discontinuation of these NNRTIs requires gradual decrease of the methadone dose over 2 to 3 weeks.	88
<b>Methadone</b>	Nelfinavir	Nelfinavir reduces the levels of methadone and its metabolites, but also decreases methadone protein binding. No change in methadone maintenance dosing was necessary in this study.	87
<b>Ethinyl Estradiol/ Norethindrone (EE/NET)</b>	Nevirapine	Nevirapine reduces EE/NET AUC by a median of 19%. Other means of primary contraception should be used in those receiving these meds together.	89
<b>Rifabutin</b>	Indinavir	Indinavir 1000 mg q8h with rifabutin 150 mg qd compensates for the AUC-lowering effect of the rifabutin on indinavir.	90
<b>Rifabutin</b>	Ritonavir/saquinavir	With ritonavir/saquinavir (400/400 mg bid), dual protease inhibitor therapy, ritonavir and saquinavir AUCs were not significantly changed by rifabutin. Reciprocal effects on rifabutin were mainly noted in the increased exposure of its active metabolite. The authors recommended 150 mg every 3 days or 300 mg every 7 days when combined with ritonavir/saquinavir.	91
<b>Mefloquine</b>	Ritonavir	Mefloquine reduces ritonavir AUC by 35%. No significant reciprocal effects were seen.	92
<b>HMG-CoA Reductase Inhibitors (Statin Lipid- Lowering Agents)</b>	Ritonavir/saquinavir	Ritonavir/saquinavir increases simvastatin median AUC by 2676%. Therefore, this combination should be avoided. The atorvastatin AUC is increased by 74%. The authors suggest cautious coadministration. Pravastatin median AUC is decreased by the dual protease inhibitors by 47% and, therefore, can be administered with these drugs. No pravastatin dose adjustment is suggested.	LB6
<b>Emivirine</b>	Efavirenz	Administered at adjusted doses of emivirine 500 mg bid and efavirenz 800 mg qd, PK parameters including the AUC, $C_{min}$ , and $C_{max}$ of both drugs were unexpectedly lowered.	670
<b>Nevirapine</b>	Efavirenz	Nevirapine causes a 22% efavirenz AUC reduction without significant reciprocal effects.	80
<b>Capravirine</b>	Indinavir or nelfinavir	Capravirine AUC increased by approximately 2-fold without reciprocal effects.	83
<b>Delavirdine</b>	Saquinavir	Delavirdine decreases saquinavir clearance by 63%, thus allowing for saquinavir to be dosed at 1400 mg bid.	82
<b>Emivirine</b>	Indinavir	Emivirine reduces indinavir AUC by 74% and indinavir increases emivirine AUC by 88.5%; therefore, emivirine should not be combined with indinavir in a single protease inhibitor regimen.	84

**Table 3. Body Compartment Pharmacokinetic Characteristics**

Antiretroviral Drug(s)	Cerebrospinal Fluid (CSF)	Semen	Abstract No.
<b>Indinavir</b>	Mean CSF AUC was 1.7% of plasma with large interindividual variability.		311
<b>Indinavir</b>	In adults receiving indinavir 800 mg q8h, CSF indinavir levels at 3 hrs best predicted CSF AUC.		308
<b>Indinavir/Ritonavir</b>	Ritonavir 100 mg bid enhanced indinavir CSF levels by 3-fold.	Ritonavir 100 mg bid enhanced indinavir semen levels by 7.5-fold.	312
<b>Lamivudine</b>	Median CSF:serum ratio was 12% but varied with the time post-dosing (at <6 h, ratio is 9.6%; >6 h, ratio is 56%). Median CSF level (890 ng/mL) was adequate.		310
<b>Emivirine</b>	Mean CSF:plasma ratio was 7.8%, yielding emivirine levels above the in vitro IC <sub>90</sub> .		315
<b>Nelfinavir/Saquinavir</b>	Nelfinavir and saquinavir were undetectable in CSF.	In 3 of 8 semen samples, nelfinavir was below detection limits, and saquinavir was detectable at low levels.	316
<b>Amprenavir/Zidovudine/Lamivudine</b>		At 6 h post-dose, semen: blood ratios for amprenavir, zidovudine, and lamivudine were 0.20, 2.29, and 5.13, respectively.	317
<b>Amprenavir/Zidovudine/Lamivudine</b>	Mean amprenavir CSF:plasma ratio was 0.0295, but concentrations exceeded the expect was IC <sub>90</sub> .		314
<b>Zidovudine/Lamivudine/Abacavir/Nevirapine/Indinavir</b>	At week 8, 1h post-dosing, the mean CSF levels (CSF: serum ratios) were: 37 ng/mL (0.04) for zidovudine, 52 ng/mL (0.06) for lamivudine, 71 ng/mL (0.06) for abacavir, 1158 ng/mL (0.26) for nevirapine, 51.5 ng/mL (0.007) for indinavir.  Despite these relatively low CSF drug levels, CSF virologic response was noted out to 48 weeks with median baseline CSF RNA levels decreasing from 3.6 to <1.6 log <sub>10</sub> copies/mL.		309
<b>Indinavir</b>  <b>Ritonavir</b>  <b>Saquinavir</b>		Indinavir had better semen penetration than ritonavir or saquinavir. Indinavir median semen: blood ratios ranged from 0.6 to 1.4 depending on the time post-dose.  Ritonavir median semen: blood ratio was 0.029.  Saquinavir median semen: blood ratio was 0.036.	318

AUC indicates area under the curve; PK indicates pharmacokinetic; IC<sub>90</sub> indicates 90% inhibitory concentration.

the saquinavir (34%), indinavir (36%), and nelfinavir (34%) arms compared with the placebo arm (23%) had plasma viral loads of less than 200 copies/mL ( $P=0.004$  for the dual protease inhibitor arms versus the single protease inhibitor arm). There were no significant differences in virologic outcome between the various dual protease inhibitor arms. Comparing NNRTI-naive versus NNRTI-experienced subjects, 43% versus 16% had viral loads of less than 200 copies/mL ( $P<0.001$ ). Notably, 47% of NNRTI-naive subjects receiving dual protease inhibitor therapy versus 8% of NNRTI-experienced subjects receiving amprenavir monotherapy had plasma viral loads of less than 200 copies/mL in follow-up. Across the 4 study arms, the CD4+ cell count increases ranged from 9/ $\mu$ L to 53/ $\mu$ L at 24 weeks. Pharmacokinetic analysis of protease inhibitors at day 14 demonstrated mean AUCs ( $\log_{10}$  transformed) that were lower in the amprenavir/placebo (4.0) and amprenavir/saquinavir (3.95) arms, compared with the amprenavir/nelfinavir (4.27) and amprenavir/indinavir (4.28) arms. Grades 3 and 4 drug-associated signs and symptoms were noted in 19%, increased triglyceride level or hypophosphatemia in 28%, grade 2 or worse rash in 17% and adefovir-associated nephrotoxicity in 19% of subjects.

Gulick and colleagues (Abstract 235) evaluated the efficacy of 5 salvage regimens in 277 subjects in whom an indinavir-based regimen had been failing for at least 6 months and who were naive to NNRTIs, saquinavir-*sgc*, nelfinavir, amprenavir, and adefovir (ACTG 359). The regimens were saquinavir-*sgc* (400 mg bid)/ritonavir (400 mg bid) with delavirdine (600 mg bid) or adefovir or both delavirdine/adevovir. A parallel component contained saquinavir-*sgc* (400 mg bid)/nelfinavir (750 mg tid) with delavirdine (600 mg bid) or adefovir or both delavirdine/adevovir. At week 16 the adefovir dose was reduced from 120 mg to 60 mg daily. The median CD4+ cell count and plasma HIV RNA were 229/ $\mu$ L and 31,746 copies/mL, respectively. At baseline, 50% had at least a 4-fold reduced susceptibility to indinavir. Of the 77 of 254 subjects (30%) with plasma viral loads of 500 or fewer copies/mL at week 16, 55% (ITT analysis,  $M=F$ ) and 70% (OT analysis) maintained this response out to

48 weeks. At 48 weeks 35 subjects had experienced adefovir-associated proximal renal tubular disorder (PRTD). The factors predictive of 16-week viral load response were a higher baseline CD4+ count (OR, 1.1/100 cells/ $\mu$ L;  $P=0.003$ ), lower baseline HIV RNA levels (OR, 0.34/ $\log_{10}$  HIV RNA copies/mL;  $P<0.001$ ), shorter duration of indinavir use (OR, 0.93;  $P=0.002$ ), and female gender (OR, 0.40;  $P=0.004$ ).

### **Trials in Multiple Antiretroviral-Experienced Subjects**

Montaner and colleagues (Abstract 536) presented data on multidrug rescue therapy (MDRT) in 2 cohorts with histories of failure of multiple prior antiretroviral regimens. In cohorts I ( $n=106$ ) and II ( $n=68$ ) the median CD4+ cell counts and plasma HIV RNA levels were 180/ $\mu$ L and 205/ $\mu$ L and 62,000 and 56,500 copies/mL, respectively. Subjects were treated with a median of 5 and 7 antiretrovirals in cohorts I and II, respectively. The treatment regimens included up to 2 protease inhibitors, 2 NNRTIs, 4 nRTIs, and hydroxyurea. In cohorts I and II, 35% and 44% of subjects had plasma HIV RNA levels of less than 400 copies/mL at 25 and 35 weeks of follow-up, respectively. In follow-up, the median CD4+ cell change for cohorts I and II were 0 and -10/ $\mu$ L, respectively. In both cohorts, the median baseline HIV RNA levels were significantly higher and the median CD4+ cell counts significantly lower in the nonresponders versus the responders. Baseline phenotypic drug susceptibility data in cohort I demonstrated some degree of resistance to 7 or more of the antiretroviral drugs in 59% of isolates tested. Susceptibility to lamivudine, didanosine, and stavudine was positively associated with achieving a virologic response ( $P<0.05$ ).

### **Baseline Phenotyping to Guide Salvage Therapy**

Two studies were presented evaluating the impact of drug susceptibility testing (phenotyping) when applied to subjects in whom the current antiretroviral regimen is failing in the clinical setting (Abstracts 237 and 786). Cohen and colleagues described an open-label, randomized study of subjects in whom

the first protease inhibitor-based regimen was failing and on-treatment plasma viral loads were more than 2000 copies/mL. The Antivirogram (Virco) drug susceptibility assay was employed in this study. In the phenotypic resistance testing and standard of care (SOC) arms, 111 and 110 subjects were enrolled, and the median CD4+ cell counts and plasma HIV RNA levels were 402/ $\mu$ L and 384/ $\mu$ L and 4.19  $\log_{10}$  copies/mL and 3.91  $\log_{10}$  copies/mL, respectively. At entry, nelfinavir was failing in 53% of subjects and indinavir in 36% of subjects, and 95%, 83%, 61%, and 31% of subjects had prior zidovudine, lamivudine, stavudine, and didanosine experience, respectively; 4% of subjects were NNRTI-experienced. Baseline susceptibility profiles demonstrated a greater than 10-fold resistance to nelfinavir, indinavir, and ritonavir in approximately 50%, 15%, and 25% of subjects, respectively. Isolates were generally fully susceptible to abacavir and resistance to stavudine was very uncommon. Among isolates with a 4-fold or greater resistance to indinavir or nelfinavir, the percentages also susceptible to amprenavir were 70% and 90% and to saquinavir were 60% and 80%, respectively. The percentages of subjects receiving 3 or more active antivirals in the phenotypic and SOC arms were 77% and 62%, respectively. At week 16, 58% and 37% of subjects (observed data) had plasma HIV viral loads of less than 400 copies/mL in the phenotypic and SOC arms, respectively ( $P=0.011$ ). Similarly, the median changes in plasma HIV RNA from baseline were -1.27 and -0.75  $\log_{10}$  copies/mL in the phenotypic and SOC arms, respectively ( $P<0.005$ ). These preliminary data demonstrate the potential utility of the phenotypic resistance testing as an aid to selection of antiretrovirals in the next treatment regimen among subjects experiencing virologic failure.

Melnick and colleagues (Abstract 786) described the results of a pilot salvage therapy trial of 115 heavily nRTI- and protease inhibitor-experienced subjects with plasma viral loads of more than 2000 copies/mL on treatment. Subjects were randomized to receive standard of care (SOC arm,  $n=61$ ) or to have salvage therapy guided by the Antivirogram (Virco) phenotypic resistance

test (PRT arm, n=54). Subjects were NNRTI-naive but had significant protease inhibitor experience, with more than 43% of subjects having prior experience to 3 or more protease inhibitors. The mean number of susceptible drugs prescribed to each subject in the SOC and PRT arms were 1.92 and 2.77, respectively (P=0.0001). At 4 weeks follow-up the mean changes in plasma HIV RNA from baseline were -0.5 and -1.0 log<sub>10</sub> copies/mL in the SOC and PRT arms (P=0.137). However, at the 16-week follow-up the prevalence of virologic failure was 69% and 85% in the SOC and PRT arms. The authors note the high virologic failure rate (70%) among subjects treated with 3 or more “sensitive” drugs and suggest that in this heavily experienced population, archived resistant virus may have reemerged on treatment.

## Prevalence of Resistance and Novel Resistance Patterns

### Prevalence of Resistance Among Antiretroviral-Experienced Subjects

Hertogs and colleagues (Abstract 740) described common and less frequent resistance patterns observed in over 5000 isolates in the Virco database. In the reverse transcriptase gene a Q151M mutation was observed in 1.5% of the isolates. Insertions at codon 69 were observed in 0.8% of isolates. Ten types of 69 insertions were observed; the most frequent were -SSS- (10/38), -SSG- (10/38) and -SST- (6/38), with 33 of 38 having a concurrent mutation at codon 215. The T215Y or F and T215D or C or V mutations were observed in 46% and 2.5% of isolates, respectively. The M184V and M184I or T mutations were seen in 42% and 1.02%, respectively. The T69D or S mutations were seen in 10/0.8% of isolates, respectively. The K103N and K103R or H or S or T mutations were observed in 20% and 2.29% of isolates, respectively. The Y181C and Y181I or V were observed in 15% and 1.1% of isolates and K101E and K101Q or H or N or P were observed in 2.5% and 3.0% of isolates, respectively. In the protease gene, the L10I and L10S or V or R mutations were observed in 31% and 7.3% of isolates, respectively. The mutations V82A and V82S or F or T were

observed in 16% and 4.3% of isolates, respectively. The D30N and L90M mutations were observed in 6% and 33% of isolates. These data emphasize the need for ongoing HIV drug resistance surveillance and highlight concerns regarding the evolving phenomenon of multidrug-resistant strains of HIV.

### Prevalence of Resistance Among Antiretroviral-Naive Subjects

Several conference abstracts dealt with the prevalence of drug resistance mutations among individuals naive to antiretroviral drugs (Abstracts 747, 748, and 749). Cassol and colleagues described a cohort of 35 HIV-seropositive subjects documented to have seroconverted within the preceding 2 years in the Canadian province of Ontario. The mean CD4+ cell count and plasma viral load was 582/μL and 4.9 log<sub>10</sub> copies/mL, respectively. While no primary protease mutations were observed, secondary mutations were seen in 22 of 35 subjects. Among the most frequent secondary mutations observed were L63P (62.5%), V77I (43%), M36I (17%), and L10I (8.7%). Mutations associated with nRTIs were seen in 3 subjects, V179D (1), V106I (1), and Y181C (1). Nucleoside-associated mutations were observed in 2 subjects, M41L (1), and M41L/L210W (1).

### Uncommon Genotypes Associated with Antiretroviral Drug Resistance

***gag-pol* Cleavage Site Mutations.** In a study of 28 patient-derived protease inhibitor-resistant isolates, insertions or mutations in the *gag-pol* cleavage sites were observed to occur frequently when compared to baseline sequences and to isolates from subjects who never received protease inhibitors (Abstract 722). Mutations at the p2/p7 and p7/p1 regions were observed in 50% and 36% of protease-resistant isolates and 11% and 0% of controls. The mutation QAN/F to QVN/F on the 5' side of the p7/p1 region was the most common and specific adaptation observed among protease-resistant isolates. Mutations outside of these regions were rare. Notably, the emergence of these mutations was not linked to the duration of protease ther-

apy, the number of sites mutated, or the number of primary protease mutations.

Peters and colleagues (Abstract 724) evaluated the changes in the p6<sup>gag</sup> region in the setting of antiretroviral therapy. Most notably a duplication of the PTAPP motif, typically to PTAP-PAPP, was observed among 28 of 114 samples from subjects in whom antiretroviral therapy was failing but was not observed among antiretroviral-naive subjects. In vitro, a recombinant isolate bearing this motif in an NL4-3 background was replication competent, and 2 such recombinants demonstrated modest 3-fold or less cross-class antiretroviral resistance.

**Novel Protease Mutations.** Several presentations highlighted 1 to 6 codon insertions occurring between protease codons 35 to 38 (wild-type amino acid sequence EMNL). Winters and colleagues (Abstract 723) described 15 such sequences from patients in whom protease-based therapy was failing and from over 900 patient-sequences analyzed. The most common motif, a duplication of neighboring codons in this region, was observed in 9 of 15 sequences. Notably, these mutations were not consistently observed to occur in association with major protease resistance mutations (6 of 15 had no major protease inhibitor mutations). While such inserts are distal to the active site of the protease they are located at the enzymatic flap subdomain. No site-directed mutagenesis-based data or phenotypic data were presented in this abstract.

**Novel Reverse Transcriptase Mutations.** Codon insertions between reverse transcriptase (RT) codons 68 and 69 in the setting of zidovudine resistance are associated with modest levels of resistance to available nucleoside analogues. Goudsmit and colleagues (Abstract 737) described the natural history of one such isolate, demonstrating the emergence of the insert after 6 months of zidovudine monotherapy and its disappearance and rapid reemergence in serum on treatment withdrawal and reinstitution, respectively. Using dynamic population modeling, the relative fitness of the insertion mutant was estimated to be 37% less than wild-type in a drug-free milieu and 55% higher in the setting of drug

Table 4. Trials in Antiretroviral-Experienced Subjects

Abstract Number	Study	Prior Antiretroviral Experience	Regimen/Study Arm	Number of Patients	Baseline Plasma HIV-1 RNA copies/mL
532	ABT-378/Ritonavir	First protease inhibitor failure (indinavir, 44%; nelfinavir, 36%; saquinavir, 13%; ritonavir, 6%) naive to 1 nRTI, NNRTI naive	ABT 378/Ritonavir  (nevirapine and nRTIs added at day 15)	70	4.0 log <sub>10</sub>
525		Stavudine or Didanosine and Lamivudine  Zidovudine/Lamivudine	Zidovudine/Delavirdine/Indinavir or Zidovudine/Lamivudine/Indinavir Stavudine/Delavirdine/Indinavir	31 33 41	3.06 log <sub>10</sub>
526	ACTG 373	Amprenavir (36) Amprenavir/nRTIs (20) NNRTI	Stavudine/Lamivudine/ Nevirapine/Indinavir (1000 mg q8h)	56	4.19 log <sub>10</sub>
529		nRTI	Indinavir/Efavirenz/Placebo  Indinavir/Efavirenz/Abacavir	143 140	4.30 log <sub>10</sub>
531	ACTG 364	nRTI	1-2 new nRTIs/Efavirenz 1-2 new nRTIs/Nelfinavir  1-2 new nRTIs/Nelfinavir/ Efavirenz	65 66 64	
LB7		nRTI NNRTI Protease inhibitor 26% had >16 weeks prior exposure to 3 protease inhibitors	Abacavir/Adefovir/Efavirenz/ Amprenavir plus  Saquinavir or Indinavir or Nelfinavir or Placebo	481	4.7 log <sub>10</sub>
456	ACTG 5025	On Zidovudine/Lamivudine/ Indinavir for 6 months  Naive to Didanosine and Stavudine	Zidovudine/Lamivudine/ Indinavir/  Stavudine/Didanosine/ Indinavir  Hydroxyurea/Stavudine/ Didanosine/Indinavir	68 68 66	100% <200 83% <50
235		nRTIs Indinavir (>6 months)	Ritonavir/Saquinavir and Delavirdine or Adefovir* or Delavirdine/Adefovir* or Nelfinavir/Saquinavir and Delavirdine or Adefovir* or Delavirdine/Adefovir*  * At week 16 Adefovir dose reduced from 120 mg to 60 mg qd	277	31,746

ITT indicates intent-to-treat analysis; OT indicates on-treatment analysis. M=F indicates that missing values equal failure.

Baseline CD4+ cells/ $\mu$ L	Follow-up (weeks)	Plasma HIV-1 RNA Change ( $\log_{10}$ ) or Percent Below Detection	CD4+ Cell/ $\mu$ L Change	Comments
349	48	60% ITT, M=F 76% OT had <50 copies/mL	+125	32% (18/57) and 19% (11/57) had 4-fold reduced susceptibility to 3 protease inhibitors and to ABT-378, respectively
511	48	77% (23/30) 39% (13/33)  48% (19/40) <50 copies/mL	+120-145	P=0.05 between zidovudine/delavirdine and zidovudine/lamivudine arms 77% (42/54) subjects continuing only 2 nRTIs had <50 copies/mL
346	48	59% ITT 78% OT  <500 copies/mL	+94	20% had plasma viral load < 500 copies/mL at baseline Subjects on amprenavir monotherapy had superior response (p <0.01) 39% stopped 1 drug 11/56 (20%) had grade 3 toxicities
133	48	69%  74%	+115  +120	No significant difference between the arms at 48 weeks (P=0.57)
	40-48	22% 44%  67%  <50 copies/mL	+94	RNA response across all arms (P=0.001, 3-way P value)  Plasma viral load <50 copies/mL at 16 weeks strongly predictive of 48-week HIV RNA response
202	24	<200 copies/mL Saquinavir arm 34% Indinavir arm 36% Nelfinavir arm 34% Placebo 23%	+9-35	P=0.004 for dual protease inhibitor versus single protease inhibitor arms 43% and 16% of NNRTI-naive and experienced patients had plasma viral load <200 copies/mL No significant differences between the dual protease inhibitor arms
617	48	3/68  6/68  7/68 experienced virologic failure		Study closed 9/99 due to excess toxicities in hydroxyurea arm including 3 deaths associated with pancreatitis
229	48	Of the 77/254 (30%) of subjects with plasma viral load <500 copies/mL at week 16  55% (ITT, M=F) and 70% (missing=excluded) had plasma viral load <500 copies/mL at 48 weeks		At baseline 50% had 4-fold reduced Indinavir susceptibility To week 48, 35 subjects had experienced proximal renal tubular disorder. Superior HIV RNA responses in those with lower viral loads and higher CD4+ counts at baseline

therapy.

Two presentations (Abstracts 740 and 741) described isolates with resistance to lamivudine in association with the RT mutations 44D or A and/or 118I. These mutations were observed to occur in the setting of zidovudine resistance and in the absence of the M184V mutation. Delaugerre and colleagues noted that these mutations may occur in the absence of lamivudine exposure and concurrently with RT codon 69 inserts and with the Q151M and M184V mutations. In the absence of M184V, these mutations are typically observed with 2 or more zidovudine resistance mutations. Hertogs and colleagues noted that isolates bearing the 44D and/or 118I mutations demonstrated lamivudine resistance only in zidovudine resistance backgrounds and that in contrast to the M184V mutation, these mutations did not restore zidovudine susceptibility.

Imamichi and colleagues (Abstract 738) described the *in vitro* characteristics of a novel nRTI resistance motif consisting of an RT codon deletion at codon 67 (67) and a codon substitution, T69G. Recombinant isolates bearing 67 or T69G remained susceptible to zidovudine while demonstrating 3.5- or 11-fold, 12- or 3.9-fold, and 11- or 7.6-fold resistance to didanosine, lamivudine, and stavudine, respectively. Isolates bearing 67/T69G with L74I, L74I/K103N, or L74I/A98G/K103N demonstrated 5.5-fold, 8-fold, and 30-fold zidovudine resistance, respectively. Notably, 67/T69G/K103N isolates had susceptibilities to available NNRTIs which were lower than those of isolates bearing the K103N mutation in isolation.

**Novel NNRTI Resistance Patterns and NNRTI Hypersusceptibility.** Whitcomb and colleagues (Abstract 234) presented *in vitro* data describing patient-derived recombinant HIV isolates with reduced nRTI drug susceptibilities but with hypersusceptibility to NNRTIs. The drug susceptibilities were measured using the PhenoSense recombinant virus assay (ViroLogic). Hypersusceptibility (n=77), susceptibility (n=100), and reduced susceptibility (n=60) were defined as fold changes of 0.4-fold or less, greater than 0.4-fold to less than 2.5-fold, and 2.5-fold or more relative to wild-type, respec-

tively. The mean number of nRTIs used previously by subjects with isolates that were hypersusceptible, susceptible and that had reduced susceptibility were 4.1, 1.2, and 1.1, respectively. Although hypersusceptibility tended to be NNRTI observed in isolates with nRTI resistance mutations, it was also observed in isolates with no recognized nRTI mutations. It is important to emphasize, however, that the relevance of these observations to the responses to treatment is as yet unknown.

Leigh Brown and colleagues (Abstract LB9) presented data highlighting codon changes in the HIV RT not previously associated with resistance to NNRTIs but that were associated with modest reductions in NNRTI susceptibilities of isolates from recently infected, untreated patients. Employing the PhenoSense assay, 13 and 19 isolates derived from 109 subjects with primary infection demonstrated modest reductions in susceptibilities to nevirapine and delavirdine, respectively. Site-directed mutants bearing the complete 83R/135T/283I motif had 4- to 5-fold reduced nevirapine, delavirdine, and efavirenz susceptibilities. Isolates bearing 135T/162S/214T or S or F were associated with 3-fold to 6-fold reduced susceptibility to delavirdine. Importantly, the distribution of these various resistance motifs across the United States did not suggest geographic clustering.

### Resistance and Cross-Resistance to Specific Drugs

**Amprenavir and Nelfinavir.** Schmidt and colleagues (Abstract 726) described the patterns of amprenavir resistance among isolates cross-resistant to 4 other protease inhibitors (indinavir, ritonavir, saquinavir, and nelfinavir). An in-house recombinant phenotypic assay was employed and susceptibilities were defined relative to a wild-type control using a more than 3-fold cut point to define resistance. Among 62 isolates demonstrating more than 3-fold reductions in susceptibility to all 4 protease inhibitors, 23 (37%) were fully susceptible (3-fold) to amprenavir, 16 (25.8%) had a 4-fold to 8-fold reduction and 23 (37%) had greater than 8-fold resistance. Furthermore, the protease resistance profiles M46I or L,

I54L or V, I84V, and L90M ( $P<0.0001$ ) and L10I or R or V or F ( $P<0.001$ ) were associated with amprenavir resistance. The presence of I84V and any 2 of M46I or L, I54L or V, and/or L90M was 88% sensitive and 79% specific in predicting greater than 8-fold reductions in amprenavir phenotypic susceptibility.

Drona and colleagues (Abstract 729) analyzed predictors of outcome among a cohort of 111 nRTI- and protease inhibitor-experienced subjects receiving nelfinavir salvage therapy, evaluated by baseline HIV phenotype (n=51) and genotype (n=111). This cohort had a median baseline CD4+ cell count and plasma HIV RNA of 208/ $\mu$ L and 4.6 log<sub>10</sub> copies/mL, respectively. Subjects were nelfinavir- and NNRTI-naïve at baseline but 69% had previously received 2 or more protease inhibitors. Subjects were treated with nelfinavir/nevirapine (32), nelfinavir/nevirapine/saquinavir (74), or nelfinavir/nevirapine/ritonavir (5). At baseline, 18 of 51 (35%), 7 of 51 (14%), and 26 of 51 (51%) of isolates demonstrated less than 4-fold, 4-fold to 10-fold, and more than 10-fold levels of nelfinavir resistance, respectively. These data correlated with plasma HIV RNA at 3 months with 40%, 14%, and 0% of susceptible, intermediate, and resistant groups having less than 200 copies/mL ( $P=0.003$ ). Furthermore, the level of phenotypic nelfinavir resistance correlated with presence of the L90M mutation ( $P=0.007$ ) and the number of protease mutations (primary and secondary) ( $P<0.001$ ). In logistic regression analysis only the number of mutations in the protease gene correlated with nelfinavir cross-resistance (RR, 2.09;  $P<0.01$ ).

**Amprenavir and Other Protease Inhibitors.** Elston and colleagues (Abstract 727) described the 48-week virologic outcomes in isolates from subjects treated with the dual protease regimens of amprenavir in combination with saquinavir (n=8), indinavir (n=9) or nelfinavir (n=7). Of the 18 subjects evaluable at 48 weeks, 13 had plasma viral loads of less than 400 copies/mL. Virologic escape was observed in 1 subject treated with amprenavir/saquinavir. In this isolate, resistance to amprenavir was associated with the presence of the L10I, L63P, I84V, and L90M mutations. In 2 subjects, virologic

failure from baseline was associated with the presence of protease inhibitor resistance mutations at baseline. Notably 2 of 8 patients on amprenavir/indinavir never responded but possessed no major resistance mutations in plasma virus at 36 and 48 weeks follow-up. The amprenavir and indinavir  $C_{min}$  values in both of these subjects were comparable to others in this group. Two-week pharmacokinetic data from this study demonstrated that when coadministered with amprenavir, the changes in the  $C_{min}$  values of saquinavir, indinavir, and nelfinavir were -48%, -27%, and +14%, respectively, relative to controls. Conversely, the changes in amprenavir  $C_{min}$  when coadministered with saquinavir, indinavir, and nelfinavir were -14%, +25%, and +189%, respectively, relative to controls.

**Efavirenz.** Wallace and colleagues (Abstract 752) presented genotypic data from a subset of subjects enrolled in DMP 266-020. This study evaluated a regimen of indinavir and 1 or 2 nRTIs with or without efavirenz in subjects with 8 or more weeks of nRTI experience and who were protease inhibitor- and NNRTI-naive. Subjects had a viral load of more than 10,000 copies/mL at baseline. The genotypic analysis was confined to those subjects in the indinavir/efavirenz/nRTI arm who had on-treatment plasma viral loads of more than 1000 copies/mL. Baseline and follow-up samples from 36 treatment failures were compared with baseline samples from 25 on-treatment responders. Among the treatment failures the sentinel K103N mutation was observed in 65% (20/31) with mutations K101Q, V108I, or P225H emerging in combination with K103N. The V82A or T mutation emerged in 3 of 31 subjects. The K103N mutation was typically observed in the first available plasma sample. However, no recognized NNRTI mutation was defined in 32% of treatment failures (follow-up range, 28 to 224 days).

**Tenofovir.** Miller (Abstract 740a) presented data describing reverse transcriptase mutations observed after 24 weeks of treatment with tenofovir disoproxil fumarate (TDF) (formerly PMPA dipivoxil) added to stable background therapy. Patients (n=189) were enrolled

in this ongoing, Phase II, blinded, dose-ranging, placebo-controlled trial. The median baseline CD4+ cell count and plasma viral load was 375/ $\mu$ L and 3.7 log<sub>10</sub> copies/mL, respectively, and mean prior antiretroviral experience was 4.6 years. Tenofovir doses evaluated were 75 mg, 150 mg, and 300 mg once daily. Among subjects taking 300 mg, a 0.75-log<sub>10</sub> copies/mL reduction from baseline was sustained out to 32 weeks of follow-up (n=105). At baseline, 97%, 59%, and 34% of subjects had genotypic evidence of resistance to nRTIs, protease inhibitors, and NNRTIs, respectively. In follow-up at week 24, 44 of 124 subjects evaluated had developed new mutations in the HIV RT, most of which were 215-complex associated and which emerged on zidovudine (n=9), stavudine (n=18), and abacavir (n=5). In 2 subjects (1.6%), the K65R mutation emerged while on abacavir or didanosine.

### Impact of Baseline Protease Polymorphisms and Mutations on Virologic Response

Para and colleagues (Abstract 732) described factors associated with 8-week virologic responses among a cohort of 89 subjects on 2 nRTIs and saquinavir hard gel capsule (hgc) (median, 105 weeks) who were randomized to continue their current therapy or to switch the protease inhibitor to saquinavir-sgc or indinavir (ACTG 333). The median CD4+ cell counts and plasma HIV RNA levels were 240/ $\mu$ L and 4.10 log<sub>10</sub> copies/mL, respectively. The most common protease mutation at baseline was L90M (59%). At week 8 the approximate mean alterations in plasma HIV RNA in the saquinavir-hgc, saquinavir-sgc and indinavir groups were +0.7, -0.24, and +0.5 log<sub>10</sub> copies/mL, respectively. Baseline factors associated with diminished HIV RNA response at week 8 were the L10I or V protease mutation and the number of protease substitutions (P<0.001). In the multivariate analysis, the phenotypic saquinavir and indinavir susceptibilities did not add significantly to the genotypic data.

Perno and colleagues (Abstract 728) highlighted the potential role of baseline polymorphisms at protease codons 10, 36, and 71 as predictors of virologic outcome in 130 protease-naive patients

commencing protease inhibitor-based therapy. At baseline, 31 (23.8%) had protease mutations at codons L10I or V or M36I and 3.1% had mutations at both positions. At 24-week follow-up, 36 (27.7%) had virologic failure defined as a plasma viral load of more than 500 copies/mL or prior treatment discontinuation because of virologic failure. Only the total number of protease mutations or polymorphisms (OR, 2.13/mutation; P=0.03) and the presence of the specific baseline protease polymorphisms L10I or V and/or M36I, in the absence of primary protease mutations, were associated with virologic failure.

Quigg and colleagues (Abstract 787) evaluated baseline RT and protease genotypes as predictors of response among a cohort of antiretroviral-adherent subjects experiencing on-treatment virologic failure followed over 24 weeks. They noted that 24-week virologic response was best predicted by the total number of primary and secondary protease inhibitor and nRTI mutations. This analysis emphasized the contribution of secondary mutations at RT codons 30, 44, 60, 135, and 202 as predictors of virologic response.

## Treatment Strategies

### Treatment Substitutions in Subjects on Stable Antiretroviral Therapy

Havir and colleagues (Abstract 456) presented data from ACTG 5025, which was designed to compare the immunologic and virologic merits of maintaining a stable regimen of indinavir/zidovudine/lamivudine (n=68) versus switching to indinavir/stavudine/didanosine (n=68) or indinavir/stavudine/didanosine/hydroxyurea (600 mg bid) (n=66). Subjects had been on indinavir/zidovudine/lamivudine for at least 6 months, were naive to didanosine and stavudine, and had plasma viral loads of less than 200 copies/mL at entry. Study endpoints included plasma HIV RNA values of 200 or more copies/mL and drug-associated toxicities. At baseline the median CD4+ cell count was 617/ $\mu$ L and prior indinavir exposure was 86 weeks. Notably, 83% had plasma viral loads of less than 50 copies/mL at entry. The study commenced in November, 1998 and was

closed in September, 1999 because of excess toxicities in the hydroxyurea arm, in which 15 of 68 subjects experienced grade 3 or higher drug-associated toxicities, including 3 deaths. In the indinavir/stavudine/didanosine and indinavir/zidovudine/lamivudine arms, 6 of 68 and 2 of 66 subjects experienced grade 3 or higher drug-associated toxicities. Furthermore, 7 of 68, 6 of 68, and 3 of 68 reached virologic endpoints in the hydroxyurea, stavudine/didanosine, and zidovudine/lamivudine arms, respectively. There were 3 cases of pancreatitis in the hydroxyurea arm and 3 in the stavudine/didanosine arm, occurring 12 to 36 months after entry. All 3 deaths, which occurred in the hydroxyurea arm, had concurrent pancreatitis. However, in 1 case the pancreatitis was reported to occur in the setting of a prior myocardial infarct and was not the immediate cause of death.

**Protease Inhibitor Substitution with Abacavir.** Two similar studies (Abstracts 51 and 457) evaluated the efficacy of continuing a stable protease inhibitor/2 nRTI regimen versus switching to an abacavir/2 nRTI regimen. Opravil and colleagues presented data from a Swiss cohort. Eligible subjects had sustained plasma HIV RNA levels of less than 50 copies/mL for 6 months or more, could not have previously failed zidovudine- or lamivudine-containing regimens, and could not have a mutation at RT codon 215 in PBMCs at baseline. At baseline, subjects in the abacavir (n=84) and protease inhibitor (n=79) arms had plasma viral loads of less than 50 copies/mL for a mean of 16 and 15 months, and median CD4+ cell counts of 513/ $\mu$ L and 522/ $\mu$ L, respectively. After a median follow-up of 48 weeks, there were 9 and 5 virologic failures in the abacavir and protease inhibitor arms, respectively. Genotyping in 7 of 9 abacavir failures demonstrated the M184V mutation in 6 of 7, and 7 of 7 subjects had a variety of zidovudine-associated mutations. There were no significant differences in the CD4+ cell count gains between the 2 arms at 48 weeks. Cholesterol levels were approximately 1 mmol/L lower in the abacavir arm than in the protease inhibitor arm ( $P < 0.0001$ ) but there were no significant differences in high-density lipoprotein

(HDL) levels. There was a trend toward lower triglyceride levels in the abacavir arm; these were nonfasting levels, however.

### Treatment Substitutions in Subjects with Virologic Failure

Gisolf (Abstract 527) presented 48-week follow-up data of an open-label, randomized controlled trial evaluating effects of nRTI intensification of ritonavir/saquinavir (n=103) or ritonavir/saquinavir/stavudine (n=99) regimens in subjects experiencing on-treatment virologic failure. Subjects were protease inhibitor- and stavudine-naïve. Intensification was permitted in both arms if the plasma HIV RNA was confirmed to be more than 400 copies/mL after 12 weeks. The median CD4+ cell count and plasma HIV RNA level in the 2 groups was 255/ $\mu$ L and 4.47 log<sub>10</sub> copies/mL, respectively. At 48 weeks, 63% and 69% (ITT analysis, M=F,  $P=0.38$ ) and 88% and 91% (OT analysis) had plasma viral loads of less than 400 copies/mL in the ritonavir/saquinavir and ritonavir/saquinavir/nRTI arms, respectively. The baseline regimen was intensified in 28 (27%) and 3 (3%) of subjects in the ritonavir/saquinavir and ritonavir/saquinavir/nRTI arms, respectively. In the ritonavir/saquinavir arm, intensification was with 1 or 2 nRTIs in 28 subjects (stavudine/lamivudine in 21/28) with 1 of 28 also adding hydroxyurea. The 3 subjects in the ritonavir/saquinavir arm who received an intensified regimen all received lamivudine only. Among those undergoing regimen intensification, 27 of 28 and 3 of 3, respectively, had plasma viral loads of less than 400 copies at the last visit. Notably, grades 3 and 4 aspartate aminotransferase/alanine aminotransferase elevations were more frequent in those randomized to stavudine (n=13) versus no stavudine (n=5) ( $P=0.05$ ) and in those who were hepatitis B surface antigen-positive (8/22 or 36%;  $P=0.001$ ).

Schulman and colleagues (Abstract 534) assessed the virologic and pharmacologic impact of intensification of a stable indinavir-based regimen with ritonavir. Subjects were ritonavir-naïve, had plasma viral loads ranging from 50 to 500,000 copies/mL, and were on a stable regimen of indinavir and 2 nRTIs for 3 or

more months. At intensification, the ritonavir and indinavir dosage used was 400 mg twice a day of each drug. Among the 35 subjects enrolled, the median plasma viral load and mean CD4+ cell count was 3.1 log<sub>10</sub> copies/mL and 424/ $\mu$ L, respectively. After entry, 7 of 35 subjects prematurely discontinued the study. At 16-week follow-up, 9 of 17 evaluable subjects had plasma viral loads of less than 50 copies/mL (median baseline plasma viral load, 243 copies/mL). In 6 of 9 subjects with available data, the median baseline indinavir C<sub>0</sub> (predose concentration) rose from 0.063 to 0.87  $\mu$ g/mL by week 3. For the complete study group (n=16) the median baseline indinavir C<sub>min</sub> and AUC<sub>24</sub> changed from 0.15 to 0.54  $\mu$ g/mL and from 47 to 51  $\mu$ g/h/mL, respectively. By week 3 there was a mean increase in CD4+ cell counts of 52/ $\mu$ L. Elevations in cholesterol (300 mg/dL) and in fasting triglyceride levels (>750 mg/dL) were recorded in 7 of 28 subjects. There were no recorded cases of nephrolithiasis or renal colic.

### Predictors of Virologic Response or Disease Progression

Mellors and colleagues (Abstract 451) investigated patterns of HIV RNA response in drug-naïve and -experienced populations initiating a new antiretroviral regimen. Among subjects experiencing virologic failure (detectable HIV RNA at week 24), 2 patterns of plasma HIV RNA response were observed. The first pattern was essentially identical to that observed in subjects with successful suppression of plasma viral load but with an abrupt rebound in plasma virus RNA. The second was marked by a slow decline in plasma viral load.

In an antiretroviral-naïve population or zidovudine-experienced population (ACTG 343) only 2% to 3% of those subsequently experiencing virologic failure demonstrated an "off-track" (slow decline) pattern within the first 4 weeks. Among the highly antiretroviral-experienced population of ACTG 359, an "off track" pattern within the first 4 weeks was more common and was observed in 40% of subjects. Furthermore, in the antiretroviral-naïve population, neither the initial

slope decline nor the baseline plasma HIV RNA was predictive of time to rebound. However, in the antiretroviral-experienced population, the baseline HIV RNA level was predictive of time to rebound and the initial slope decline was not.

Among antiretroviral-naive subjects with baseline plasma HIV RNA of less than 5.0 log<sub>10</sub> copies/mL, the median time to suppression of plasma HIV RNA to less than 200 copies/mL in 75% of subjects was 8 weeks. Conversely, among antiretroviral-experienced subjects with plasma viral loads greater than 5.0 log<sub>10</sub> copies/mL, the median time to suppression of plasma HIV RNA to less than 200 copies/mL in 75% of subjects was 12 weeks.

Guerin and colleagues (Abstract 452) compared the impact of CCR5 32-deletion heterozygosity with wild-type in achieving and maintaining a response to potent antiretroviral therapies among a cohort of protease inhibitor-naive subjects. Of the 166 subjects enrolled, 22 (13%) were heterozygous for the 32 deletion and 144 were wild-type. At 6 months follow-up, 82% and 49% of subjects in the 32 and wild-type groups, respectively, had a virologic response (viral load <500 copies/mL or a 2 log<sub>10</sub>-decrease in viral load from baseline) and an immunologic response (increase in CD4+ cells of 50/μL) (P<0.01). Furthermore, these differences were maintained out to 12 months.

Miller and colleagues (Abstract 454) evaluated factors associated with disease progression and death in 1255 subjects with at least 1 CD4+ cell count below 50/μL in the EuroSIDA cohort. Subjects were followed until diagnosis of a new AIDS-defining illness or until the CD4+ cell count rose to above 50/μL. At baseline, the proportion of subjects who were treatment-naive, treatment-experienced but off-therapy, and on-treatment were 5%, 10%, and 85%, respectively, with 60% being on 3-drug therapy. The median CD4+ cell count and plasma HIV RNA was 25/μL and 4.73 log<sub>10</sub> copies/mL. In follow-up, there were 292 events including 177 (44%) new AIDS-defining illnesses and 90 (30%) deaths. Relative to subjects on no therapy, the event rate ratio for subjects on 3 drugs was 0.4. In multivariate modeling the relative hazard

for disease progression or death in subjects receiving protease inhibitor-based therapies was 1.22 per 50% CD4+ cell decline (P<0.0001), 1.3 per log<sub>10</sub> lower plasma viral load (P<0.0001). Overall, protease inhibitor-based therapy was associated with a 38% reduction in the incidence of disease progression or death independent of the CD4+ cell count or the plasma viral load.

### **The CD4+/HIV RNA Dissociation or Disconnect Phenomenon**

Deeks and colleagues (Abstract 236) presented preliminary follow-up data from the San Francisco cohort evaluating the relative preservation of CD4+ cell counts in the setting of established virologic failure among 482 subjects treated with protease-based potent antiretroviral regimens. In 340 subjects with available pretherapy CD4+ count and viral load data, a correlation was noted between the mean change in plasma viral load over the first 6 months of treatment and the mean change in CD4+ cell counts at 6 months (rho -0.37; P=0.01).

Virologic failure was observed in 267 (55%). At the time of virologic failure the median CD4+ count and plasma HIV RNA was 153 cells/μL and 4.55 log<sub>10</sub> copies/mL, respectively. The median prior duration of nRTI treatment was 44 months. Fifty-eight patients (12%) were lost to follow-up and 49 (10%) died. Salvage therapy had been successful in 56 (21%) and these subjects were censored, as were subjects who stopped all therapy for 16 weeks. At follow-up the median duration of virologic failure was 36.7 months. Excluding censored subjects, 40% had a return of CD4+ cell counts to baseline by 36 months. In multivariate logistic regression analysis, both the mean absolute HIV RNA level over the first 6 months of virologic failure and the mean decrease in HIV RNA relative to pretherapy baseline over the first 6 months were predictive of time to return of CD4+ cells to baseline. Thus, patients with smaller reductions in plasma HIV RNA relative to pretherapy baseline had a faster rate of return of CD4+ cells to baseline. The rate of return of CD4+ cell counts to baseline was also greater among those with higher baseline CD4+ cell counts.

The rates of return to baseline of

CD4+ cell counts in this treated population were compared to matched subjects from a historical cohort of untreated controls (San Francisco Men's Health Study Reference Group). The viral load levels at 12 weeks, subsequent to onset of virologic failure, were used for comparison. This comparison demonstrated that across viral load strata, ranging from 3000 to more than 30,000 copies/mL, the rates of depletion of CD4+ cells were significantly greater among untreated subjects compared to matched subjects experiencing treatment failure (P<0.05).

Deeks and colleagues (Abstract 156) evaluated the relative growth patterns of recombinant HIV isolates derived from the plasma of 2 groups of subjects, virologic nonresponders (sustained viral loads of more than 2500 copies/mL for a median of 26.8 months on protease inhibitor-based therapy, n=24) and untreated patients (n=12). Using the ViroLogic PhenoSense assay, a recombinant HIV-1 vector was used, incorporating a luciferase indicator gene and the patient-derived reverse transcriptase and protease sequences. With drug-free conditions, the replication of each recombinant isolate was compared to a wild-type control (NL4-3) run separately, and a relative replicative index was defined with a value of less than 1 representing impaired replication relative to the control.

Median plasma viral loads for the nonresponders and untreated subjects were 4.3 and 4.8 log<sub>10</sub> copies/mL, respectively. The nonresponders had a median CD4+ cell count of 119/μL above the pretherapy nadir and had a mean 58-fold decreased susceptibility in their protease inhibitor therapy. The median replication fitness values for the nonresponders and the untreated patients were 0.2 and 1.2 (P=0.0001). Relative to the untreated state, the virologic failure group experienced an overall reduced level of CD4+ cell activation as measured by CD4+ CD38+DR+ (P=0.001) and a reduced level of CD4+ T cell turnover as measured by CD4+ Ki67 nuclear antigen (P=0.02).

The effects of treatment interruption among a subset of this population were described by Deeks and colleagues (Abstract LB10). These subjects had experienced virologic failure of a protease inhibitor-based regimen for 1 year or more, with plasma viral loads of 2500 or more

copies/mL on stable treatment for 16 weeks. After treatment interruption subjects were evaluated weekly and those experiencing a greater than 1 log<sub>10</sub>-rise in plasma HIV RNA and/or a 100 or more cells/μL drop were encouraged to restart therapy. Eighteen patients were evaluated with a median plasma viral load of 4.6 log<sub>10</sub> copies/mL and a median CD4+ cell count of 245/μL. In 17 of 18 patients, phenotyping revealed a mean 56-fold reduction in susceptibility to the protease inhibitor used at entry. In follow-up 12 weeks after discontinuing drug, the mean CD4+ cell and plasma HIV RNA changes from baseline were -94/μL and +0.82 log<sub>10</sub> copies/mL, respectively. In 16 patients after withdrawal of antiretroviral therapy, the observed high-level protease resistance was replaced suddenly over a 1 to 2 week period by a susceptible phenotype. However, the timing of this change was very variable among subjects (5.8 to 11 weeks). The emergence of the susceptible virus in plasma was associated with a sudden increase in plasma HIV RNA and fall in CD4+ cell counts. Cultures of patient PBMC pairs (before and after the shift from resistant to susceptible phenotype) demonstrated that the resistant virus could still be cultured from PBMCs in 4 of 8 subjects when tested in the presence of the appropriate protease inhibitor. The associated genotype was not detected in concurrent plasma specimens by population- and clonal-based sequencing methods, indicating that the prevalence of the resistant population was less than 10%.

The mechanisms of the relative preservation of CD4+ cell numbers in the setting of established protease inhibitor failure are as yet undefined. Penn and colleagues (Abstract 155) evaluated the growth and cytopathogenicity of HIV strains demonstrating high-level protease resistance as determined by genotype in competition cultures with wild-type virus in the presence and absence of ritonavir. The cells used were human lymphoid histocultures derived from tonsillar biopsies. Isolates originated from patients experiencing protease inhibitor failure but who had stable or increasing CD4+ cell counts. Specifically, the isolates were derived from PBMCs or were recombinant isolates bearing the protease gene derived

from the patient's plasma in an NL4-3 background, yielding either syncytium-inducing (SI) or non-SI (NSI) strains. The protease-resistant isolates employed demonstrated I54V/V82A (in SI and NSI backgrounds) or I01/46/63/77/82T/84V and I01/20R/36I/71V/82A/84V/90M (both SI strains). Relative to wild-type, these protease-resistant strains demonstrated relatively efficient replication in the presence and absence of ritonavir. Furthermore, these strains demonstrated the ability to deplete CD4+ cells equivalently to wild-type virus. Thus, a cell system derived from lymphoid tissue containing relatively mature cells may support the growth of protease inhibitor-resistant viruses. In addition, impaired CD4+ cell cytopathogenicity is unlikely to be the principal mechanism for the relative preservation of CD4+ cell counts in subjects with established virologic failure of protease inhibitor-based therapies.

Conversely, Lecossier and colleagues (Abstract 453) evaluated the thymic output of CD4+ cells in 16 subjects with established virologic failure of a protease inhibitor-based regimen. Treatment was continued for 1 or more years after initial protease inhibitor failure. Subjects had viral loads greater than 3.0 log<sub>10</sub> copies/mL and had a 1-log<sub>10</sub> or fewer copies/mL change from baseline at entry. Data presented were from follow-up at 2 years. Thymic output was estimated by quantitation of T-cell receptor excision circles (TRECs). Relatively higher numbers of TRECs in CD4+ cells reflect recent thymic activity. Quantitating TRECs by real-time polymerase chain reaction (PCR) and correcting for the number of CD4+CD4+5RA+ (naive) cells demonstrated a significant correlation with absolute CD4+ cell numbers (RR=0.51; P<0.002). Furthermore, these levels tended to be higher in subjects with increases in CD4+ cell counts above 200/μL than in those with counts below 200/μL. Correlations between TREC levels and relative viral fitness (RR=-0.36; P=0.02) and between the change in CD4+ cell count and age (P<0.02) were also noted. However, TREC levels were not found to be associated with plasma HIV RNA levels. These data suggest that factors specific to the thymic milieu, eg, local viral fitness, may be relevant to the maintenance of CD4+ cell counts in this setting.

### Viral Replication in Subjects with Stable Suppression of Viral Load

D'Aquila and colleagues (Abstract 238) presented data from 12 patients evaluated for ongoing evolution of viral resistance in the setting of sustained suppression of the plasma HIV viral load to less than 50 copies/mL. Viral isolates were obtained from PBMC culture and clonal sequencing analysis was performed using biologically cloned viruses or molecular clones from culture supernatants. In a subset of these patients (5/12) during intensive follow-up, transient low-level increases in plasma viral loads of more than 50 copies/mL (so-called viral load "blips"), were observed at time points prior to the detection of viral drug resistance mutations in PBMC culture. All 5 subjects were on their second treatment regimen. Drug resistance mutations in the RT and protease sequences were identified as emerging in these 5 subjects. One patient with baseline protease mutations at codons 82/54/63/77 was demonstrated to develop L90M/L10I/71V mutations after plasma HIV RNA blips. Among patients without HIV RNA blips, no evolution of drug resistance mutations was observed. Slow declines in plasma HIV RNA and blips in plasma HIV RNA were suggested to be more likely to be associated with more underlying HIV replication.

Frenkel and colleagues (Abstract 754) described alterations in PBMC proviral DNA sequences of HIV *pol* and *env* sequences in 10 subjects with sustained suppression of plasma HIV RNA to less than 50 copies/mL over a 1.5 to 2 year treatment period. Sampling of PBMCs was performed every 6 months and limiting dilution of PBMCs was used to obtain appropriate proviral DNA samples (yielding less than 3 of 10 positive PCR reactions). Over time, 2 broad patterns were observed: a gradual evolution of sequences toward wild-type (including a loss of prior resistance mutations), and an evolution of drug resistance mutations associated with subsequent rebound in the HIV viral load. These data suggest that in some patients with viral loads of less than 50 copies/mL, active viral replication can continue and that this may be associated with the emer-

gence of drug-resistant isolates and treatment failure.

## Conclusions

The conference once again demonstrated itself to be the premier international meeting during which the state-of-the-art in antiretroviral therapy was presented. Cautious optimism regarding the development of new antiretroviral agents and the utility of resistance testing as a clinical tool was apparent, as were the limitations of our currently available

drugs and combination regimens. Overall, the conference once again echoed the basic theme that advances in drug development and the application of new monitoring tools need to be linked to advances in our understanding of disease pathogenesis if sustained and durable progress in antiretroviral management is to be made.

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