UPDATE ON DEVELOPMENTS IN ANTIRETROVIRAL THERAPY

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Studies detailing the recent advances in antiretroviral chemotherapy comprised a major component of the 5th Conference on Retroviruses and Opportunistic Infections. Among the most important positive themes were the durability of potent regimens that can be achieved with currently available combination regimens, their immunologic and clinical benefits, the marker comparability of more simplified dosing schedules compared with standard dosing regimens, the increasing permutations of effective combinations, and the promise of new agents. Amidst the generally positive and optimistic messages, cautious notes were also sounded. These included the incomplete response rate to current combination regimens, the threat of cross-resistance within existing drug classes, the urgent need for effective salvage regimens, the negative results of the initial trials of induction-maintenance strategies, and the emergence of longer-term toxic effects in individuals exposed to protease inhibitors. The balance reflected at the meeting cast an appropriate perspective on the rapidly changing field of HIV care and clinical research.

INITIAL THERAPEUTIC OPTIONS

Protease Inhibitor-Based Options

Protease inhibitor/nRTI therapy.— While several studies elucidated the pharmacokinetic, efficacy, tolerability, and resistance patterns of investigational protease inhibitors, numerous studies of the use of approved protease inhibitors (ie, saquinavir, ritonavir, indinavir, nelfinavir) were also presented. These data sets include (1) extended follow-up of previously reported clinical trials; (2) clinical efficacy comparisons of regimens based on protease inhibitors combined with nucleoside analogue reverse transcriptase inhibitors (nRTIs) in less frequent dosing schedules; (3) efficacy of a new formulation of saquinavir (soft gelatin capsule, SGC); and (4) unique protease inhibitor-specific adverse effects and drug interactions.

Extended Clinical Trial Follow-Up

The 84-week follow-up of the Merck 039 study was presented by Hirsch et al **(Abstract 383).** This was a randomized double-blind trial that enrolled zidovudine-experienced patients with CD4+ cell counts <50/μL (median, 15/μL) and a median plasma HIV-1 RNA level of 89,510 copies/mL and compared indinavir/zidovudine/lamivudine with indinavir or zidovudine/lamivudine for 24 weeks after which time all patients received open-label indinavir/zidovudine/lamivudine. At week 84, patients who were originally randomized to the 3-drug combination had a median plasma HIV-1 RNA reduction of 1.98 log, versus 1.35 log and 1.32 log reductions for those who had received initial indinavir monotherapy or dual nRTI therapy, respectively.

Clendeninn et al presented an analysis of the extended follow-up of the Agouron 511 protocol **(Abstract 372).** This study enrolled antiretroviral-naive patients with a mean baseline plasma HIV-1 RNA level of 4.9 $\log_{10}$ and a CD4+ cell count of 283 cell/μL. After 12 months, nelfinavir, administered at the standard 750 mg po tid dose combined with zidovudine and lamivudine resulted in a mean 2.9 log reduction in plasma viral level. Of these subjects, 62% had decreases in plasma HIV-1 RNA levels to below 50 copies/mL.

In the AVANTI 3 clinical trial, nelfinavir/zidovudine/lamivudine was compared with zidovudine/lamivudine in antiretroviral-naive patients with baseline CD4+ counts between 150 and 500 cells/μL **(Abstract 8).** After 28 weeks of therapy, the median log area-under-the-curve-minus baseline (AUCMB) value for the triple-drug arm was statistically greater than for the dual nRTI arm (1.85 log reduction versus 0.98 log reduction). Approximately 50% of the former group had reductions in plasma levels to below 40 copies/mL versus about 10% of the latter group. Markowitz et al also presented data on the efficacy of nelfinavir/zidovudine/lamivudine **(Abstract 371)** in a group of antiretroviral-naive subjects with mean baseline CD4+ cell count and plasma HIV-1 RNA level of 258/μL and 5.32 log respectively. This regimen resulted in a mean increase in total CD4+ cell counts of 160/μL. At 24 months, 11 of 12 evaluable subjects had a plasma HIV-1 RNA level below 500 copies/mL. Nelfinavir trough levels measured in the first year of therapy were not found to be predictive of the duration of response.

Katlama et al presented data from the ALTIS-PLUS study, an extension of the ALTIS 1 and 2 clinical trials that evaluated the 24-week efficacy of stavudine/lamivudine in antiretroviral naive and experienced subjects, respectively **(Abstract 376).** After the initial ALTIS 1 and 2 study period, ritonavir was added to patients' regimens if their plasma HIV-1 RNA level was greater than 3000 copies/mL. At week 34, 75% and 50% of subjects who added ritonavir to their stavudine/lamivudine regimen had decreases in plasma HIV-1 RNA levels to

below 3000 and below 200 copies/mL, respectively. However, by week 50, these percentages diminished to 48% and 35%, respectively, suggesting that the simple addition of ritonavir to a prior regimen failed to confer a durable virologic response in most subjects.

Comparisons of Protease Inhibitor/ Dual nRTI Regimens

Comparisons of different protease inhibitors combined with the same dual nRTI regimen included an open-label randomized trial presented by Martinez et al. The study compared the efficacy of indinavir, ritonavir, or hard-gelatin capsule saquinavir, each in combination with stavudine/lamivudine in patients in whom nRTI therapy had failed **(Abstract 370).** After 6 months, the mean log reductions in plasma HIV-1 RNA levels were 1.7, 2.2, and 1.3, respectively. In these three groups, 50%, 60%, and 30% had decreases in plasma HIV-1 RNA levels to below 200 copies/mL, respectively. While the saquinavir-based regimen was statistically less effective, it was better tolerated than the regimens containing indinavir or ritonavir. In a related study by Clumeck et al, there was no statistically significant difference between protease inhibitor-naive subjects who received indinavir or who received ritonavir in virologic, immunologic, or the endpoints of survival and AIDS-defining illness **(Abstract 386).** Similarly, in the CHEESE study, a multicenter open-label randomized trial among patients with a mean baseline CD4+ cell count of 300μL and a baseline plasma HIV-1 RNA level of 4.95 log, there was no significant difference in virologic response between the group taking zidovudine/lamivudine/indinavir and the group taking zidovudine/lamivudine/saquinavir soft gelatin capsule **(Abstract 387b).**

Various studies also compared the relative efficacies of specific dual-nRTI regimens paired with the same protease inhibitor **(Abstracts 378, 379, 380, 381).** These trials reported similar virologic responses using stavudine/lamivudine,stavudine/didanosine, or zidovudine/lamivudine combined with indinavir.

Efficacy of Newer Formulations and Agents

In an attempt to create dosing schedules that might enhance adherence, studies were conducted of protease inhibitor-based combinations comparing bid with tid schedules of administration. Nelfinavir, administered at 1250 mg bid or 750 mg tid combined with stavudine/lamivudine or zidovudine/lamivudine was assessed in two studies **(Abstracts 373, 387a).** Virologic and immunologic responses up to 32 weeks of therapy were comparable among the dosing groups, as was tolerability. Similarly, indinavir dosed at either 1000 mg or 1200 mg bid yielded at least comparable virologic responses and adverse event rates, compared with 800 mg tid, combined with zidovudine/lamivudine at week 32 **(Abstract 374).**

Eron et al compared the clinical efficacy of the recently FDA approved fixed-dose formulation of zidovudine/lamivudine with conventionally formulated, separate zidovudine and lamivudine (the regimens included a protease inhibitor). Virologic responses were similar between the study groups **(Abstract 387c).**

Data were presented using the recently approved soft gelatin capsule (SGC) formulation of saquinavir, which possesses a 10-fold increased oral bioavailability over the original formulation. The virologic and immunologic effects of the initial hard gelatin capsule formulation and the soft gelatin version were compared, with each group also taking two nRTIs **(Abstract 368).** After 16 weeks of therapy, 47% of the saquinavir SGC-assigned group versus 28% of the hard gel capsule group had reductions in plasma HIV-1 RNA levels to below 50 copies/mL from the baseline mean of 4.8 log. In another study, saquinavir SGC combined with zidovudine/lamivudine resulted in a median reduction in plasma HIV-1 RNA levels of 3.31 log; 70% had <20 copies/mL at week 32 **(Abstract 369).**

Dual Protease Inhibitor Therapy

The rationale for antiretroviral therapy with two protease inhibitors includes additive or synergistic antiretroviral effects; favorable pharmacokinetic interactions resulting in less intensive dosing schedules; and where possible, non- or partially overlapping resistance patterns. Pharmacokinetic, safety, and efficacy data related to the use of a number of dual protease inhibitor regimens were presented at the Conference.

Ritonavir/saquinavir.—Studies that examined the safety, efficacy, and durability of this, the most studied of dual protease inhibitor combinations, included a presentation by Cameron et al of a dose-ranging open label study of 141 antiretroviral naive individuals with CD4+ cell counts between 100 and 500 cells/μL who received ritonavir/saquinavir with or without 2 nRTIs (19% added 2 nRTIs) **(Abstract 388).** After 60 weeks of therapy, 89% of patients remaining on study decreased their plasma HIV-1 RNA levels to < 200 copies/mL from a mean pretreatment of approximately 4.5 log copies/mL. The 400-mg bid dosing for both the ritonavir and the saquinavir was the best tolerated of the regimens tested. The most common adverse events were liver enzyme and triglyceride elevations. In a second study by Gisolf et al, ritonavir/saquinavir was compared with ritonavir/saquinavir/ stavudine in protease inhibitor- and lamivudine-naive

subjects with a mean pretreatment plasma HIV-1 RNA level of 4.3 log and CD4+ cell count of 260 cells/μL **(Abstract 389).** At week 24, a trend favoring the 3-drug regimen was seen with 87% in the 3-drug study group having plasma HIV-1 RNA reductions to <400 copies/mL compared with 64% of those in the dual-protease inhibitors alone group. At week 12, a subanalysis of cerebrospinal fluid (CSF) viral load responses showed that 1 of 3 patients who received ritonavir/saquinavir had viral RNA levels in the CSF reduced to below the level of detection, compared with 4 of 5 of the patients who received ritonavir/saquinavir/stavudine. A notable pharmacokinetic finding was that both ritonavir and saquinavir CSF levels were unmeasurable.

Indinavir/nelfinavir.— Results from a clinical trial employing a twice-a-day dosed indinavir/nelfinavir regimen (1000 mg and 750 mg, respectively) were reported by Kerr et al **(Abstract 393).** In this study, protease inhibitor-naive subjects with pretreatment plasma HIV-1 RNA levels above 30,000 copies/mL and CD4+ counts above 100 cells/μL were enrolled. In a pharmacokinetic analysis of this combination compared with historical indinavir and nelfinavir monotherapy data, the indinavir/nelfinavir combination resulted in similar indinavir systemic exposure, but lower nelfinavir trough levels. Increasing the nelfinavir dose to 1000 mg bid improved this deficiency. At week 32 there was a median CD4+ cell increase of 133 cells/μL; in 10 of 21 patients the plasma HIV-1 RNA level was <400 copies/mL and in 6 of 10 it was <50 copies/mL. The most common adverse effect was diarrhea, which occurred in 6 patients.

Nelfinavir/ritonavir.— Gallant et al presented preliminary data on the safety and efficacy of nelfinavir/ritonavir in protease inhibitor-naive patients. The median baseline plasma HIV-1 RNA level was 32,459 copies/mL and the median CD4+ cell count was 325 cells/μL **(Abstract 394a).** Ritonavir was dosed at 400 mg bid and nelfinavir at either 500 mg or 750 mg bid. Diarrhea was the most common adverse effect, occurring in 9 of 20 patients. At week 16, reductions in plasma viral levels of greater than 2 log were seen.

Nelfinavir/Saquinavir SGC.— Two studies reported on the use of nelfinavir/saquinavir SGC in protease inhibitor-naive individuals. Opravil, on behalf of the SPICE Study Team, presented the 32-week results of a clinical trial that examined the efficacy of nelfinavir 800 mg tid/saquinavir SGC 750 mg tid, with or without 2 nRTIs relative to either of these two protease inhibitors combined with nRTIs in subjects with a mean pretreatment plasma HIV-1 level of 4.7 log **(Abstract 394b).** After 32 weeks of therapy, 36% of patients randomized to receive the 4-drug regimen had plasma HIV-1 RNA levels <50 copies/mL, compared with 40% in the nelfinavir/saquinavir SGC alone group, and 20% of those who received either nelfinavir or saquinavir SGC combined with 2 nRTIs. A separate study evaluated nelfinavir/saquinavir SGC at the same dosing schedule with up to two nRTIs. The group of 14 subjects had a median pretreatment plasma HIV-1 RNA level of 39,917 copies/mL and a median CD4+ cell count of 327/μL; there was a median log decrease in HIV-1 viremia of 2.4 at week 52 **(Abstract 394c).**

Protease Inhibitor Drug Interactions and Adverse Effects

Notable protease inhibitor pharmacokinetic interactions described at the Conference are summarized in the table.

Protease Inhibitor-Related Adverse Effects

Reported adverse effects associated with protease inhibitors included several descriptions of altered lipid and carbohydrate metabolism **(Abstracts 407-416).** This syndrome is characterized by focal fat redistribution, hyperlipidemia, and insulin resistance. It may occur earlier and more severely in patients who are taking dual protease inhibitor (ie, ritonavir/saquinavir) regimens **(Abstracts 407-414).** The frequency of this lipodystrophy is not yet clear. Protease inhibitor-related hepatitis and renal interstitial fibrosis were also reported **(Abstracts 417, 418).**

NNRTI-containing Regimens

Numerous studies provided pharmacokinetic, resistance, and clinical efficacy data related to the use of nonnucleoside reverse transcriptase inhibitors (NNRTIs) in combination with dual nRTIs or with protease inhibitor/nRTI regimens.

Delavirdine.— In a mostly antiretroviral naive study group with a mean pretreatment plasma HIV-1 RNA level of 4.5 log and CD4+ cell count of 354 cells/μL, Sargent et al reported that a regimen consisting of delavirdine/zidovudine/lamivudine resulted in a mean plasma viral RNA reduction of >2 log through week 32, with 60% of recipients having their HIV-1 RNA levels below 400 copies/mL; 50% of these subjects had levels below 40 copies/mL. Achieving a level below the 40 copies/mL limit of detection correlated better with long-term viral suppression than did reaching below

Table. Pharmacokinetic Interactions with Protease Inhibitors

Protease inhibitor	Second antiretroviral drug	Interaction	Reciprocal interaction	Abstract number
Ritonavir	Delavirdine	No significant delavirdine AUC change	Ritonavir AUC increased by 70%	340
Nelfinavir	Delavirdine	Delavirdine AUC reduced by 40%	Nelfinavir AUC increased by 100%, but hydroxy-nelfinavir (active metabolite) AUC decreased by 50%	345
Nelfinavir	Nevirapine	No significant nevirapine AUC change	Differing results of nevirapine effects on nelfinavir AUC in two studies: either unchanged **(Abstract 351)** or decreased by 46% **(Abstract 350)**	350, 351
Nelfinavir	Saquinavir hard gelatin capsule	Increased saquinavir AUC by 13-fold	Not reported	352, 353
Nelfinavir	Saquinavir soft gelatin capsule	Saquinavir AUC increased by 392%	No significant changes to nelfinavir AUC noted	354
Indinavir	Saquinavir soft gelatin capsule	Saquinavir AUC increased by 620%	No significant changes to indinavir AUC noted	354
Ritonavir	Nelfinavir	Nelfinavir AUC increased by 150%	No significant changes to ritonavir AUC	394a
Ritonavir	Saquinavir soft gelatin capsule	Saquinavir AUC increased by 20-fold	No significant changes to ritonavir AUC note	354

AUC indicates area-under-the-curve.

the 400 copies/mL threshold **(Abstracts 694, 699).**

Delavirdine susceptibilities were determined from ACTG 261, a randomized, double-blind clinical trial that compared delavirdine/zidovudine/didanosine; delavirdine/zidovudine; and delavirdine/didanosine **(Abstract 706).** Resistance to delavirdine was delayed in the triple-drug arm, relative to the dual-drug arms. Moreover, genotypic resistance patterns varied with the addition of the nRTIs. For example, the Y181C mutation developed only in the isolates from subjects in the delavirdine/didanosine arm; the P236L mutation was seen occasionally and transiently in patients in the delavirdine/zidovudine group; the K103N mutation occurred with even distribution among the three study arms.

Nevirapine.— Initial therapy with nevirapine-containing regimens was evaluated in a number of studies. The long-term follow-up of subjects from the INCAS trial demonstrated continued marker responses (median plasma HIV-1 RNA decrease of 2.55 log and CD4+ cell increase of 151/μL) for more than 130 weeks **(Abstract 695).** In a separate study, nevirapine/stavudine/lamivudine treatment was evaluated in a group of 25 antiretroviral-naive patients with a mean pretreatment plasma HIV-1 RNA level of 160,000 copies/mL and CD4+ cell count of 259 cells/μL. Through week

44, 88% of subjects remaining in the study had plasma HIV-1 RNA levels <400 copies/mL **(Abstract 696).** A study of nevirapine/indinavir in antiretroviral-naive patients with a mean pretreatment CD4+ cell count of 342 cells/μL and plasma HIV-1 RNA level of 4.8 log was presented by Beach et al. Investigators noted >2 log decrease in plasma viral load at week 24 **(Abstract 428).**

The marker and histologic responses resulting from the use of nevirapine in combination with indinavir/zidovudine/lamivudine in antiretroviral-naive subjects were evaluated by Polis et al **(Abstract 394).** In a small study group of 7 patients, plasma HIV-1 RNA level reductions between 1.5 and 3.5 log were documented within 15 days after treatment initiation. Abnormal lymph node architecture also was noted to improve with treatment as evidenced by re-established germinal centers.

nRTI-Based Therapy

Although alone they are no longer considered standard therapies, dual nRTI combinations are pivotal components of three- and four-drug regimens. As such, several studies demonstrated characteristics of specific dual nucleoside combinations that have relevance to current combination therapy. Kuritzkes, on behalf of the ACTG 306 Study Team, reported 48-week data showing that there was no statistical difference between the virologic effects of zidovudine or stavudine combined with lamivudine (1.01 versus 1.08 log reduction in plasma HIV-1 RNA, with 12% versus 4% of subjects achieving plasma viral loads <50 viral copies/mL, respectively) in antiretroviral-naive subjects. The mean pretreatment viral load of the group was 4.01 log copies/mL **(Abstract 1).** Moreover, the addition of lamivudine did not increase antiretroviral effects of didanosine monotherapy.

In agreement with previously reported in vitro antagonism between zidovudine and stavudine, Havlir and her colleagues of the ACTG 290 and 298 Study Teams concluded that zidovudine/stavudine resulted in comparable or inferior responses compared with stavudine monotherapy; the combination therefore, should be avoided **(Abstract 2).** Of note, it was also observed that prior zidovudine use diminished the efficacy of subsequent stavudine use and that didanosine resulted in a greater plasma HIV-1 RNA decrease than stavudine over a 48-week period (a 0.44 log reduction versus a 0.13 log increase, respectively).

Cellular Inhibitor Combinations: Hydroxyurea Combinations

Further encouraging data regarding the use of hydroxyurea, a cellular ribonucleotide reductase inhibitor, in combination with nRTIs and/or protease inhibitors were presented. In a late-breaker session, Lori et al reported the results of a study using a combination of hydroxyurea/indinavir/didanosine in primary HIV infection. A potentially important mechanism of action of hydroxyurea in this clinical setting may be to block cell activation and thus limit viral targets and viral production. After a mean follow-up time of 11.3 months, all 24 patients in the trial had plasma HIV-1 RNA levels below 500 copies/mL, from a mean pretreatment level of 455,700 copies/mL for the group **(Abstracts LB11, 655).** On this regimen, total CD4+ cell counts increased by a mean of 168/μL; naive CD4+ cell counts increased significantly as well. Intriguingly, after withdrawal of the drug, plasma HIV-1 RNA rebound was not seen in one of these patients for more than 12 months, although infectious virus was recoverable from this individual.

Several groups presented the virologic and immunologic effects of hydroxyurea combined with stavudine and didanosine **(Abstracts 653, 654,656,657).** Rutschmann et al employed this regimen in 144 subjects (75% were antiretroviral naive; the mean pretreatment plasma HIV-1 RNA level was 4.5 log and CD4+ cell count was 370/μL). Subjects were randomized to receive stavudine/didanosine plus hydroxyurea or placebo **(Abstract 656).** After 12 weeks, significantly more subjects who received hydroxyurea had less than 200 and less than 20 copies of HIV RNA/mL of plasma. In terms of adverse effects, in a separate study, reversible neutropenia developed in patients who had pretreatment absolute neutrophil counts below 1700/μL **(Abstract 653).**

INVESTIGATIONAL DRUGS

Investigational nRTIs

Abacavir (1592U89).— A number of studies were presented that further characterized the pharmacokinetics, safety, resistance profile, and efficacy of abacavir, a highly active carbocyclic guanosine analogue.

Pharmacokinetic studies revealed that abacavir is primarily metabolized through nonmicrosomal pathways to glucuronide and carboxylic acid metabolites and, therefore, should not interact with the cytochrome P450-mediated metabolism of protease or non-nucleoside reverse transcriptase inhibitors **(Abstract 634).** Adequate abacavir CNS penetration was suggested by Ravitch et al who demonstrated mean cerebrospinal fluid (CSF) levels that were twice the 50% inhibitory concentration (IC50) of the drug **(Abstract 636).** Among adverse effects associated with abacavir, the most significant is a hypersensitivity reaction, noted in approximately 3% of cases. It has a median onset of 9 days (range, 3-42 days) after initiation

of the drug **(Abstract 4).** This reaction is characterized by fever, malaise, nausea and vomiting, myalgias, arthralgias, and occasionally, diarrhea. Rash is common but is not always seen. Liver enzyme and creatine kinase elevations have also been noted. These symptoms typically resolve within 1 to 2 days after the drug is discontinued. If this syndrome is suspected, abacavir should be discontinued, and rechallenge with the drug is contraindicated because of the risk of a life-threatening reaction.

In work presented by Tremblay et al, this compound maintained activity in vitro against zidovudine-resistant HIV-1 isolates (albeit, 2- to 5-fold higher IC_{50} than in zidovudine-susceptible isolates.) synergy with zidovudine, nevirapine, and amprenavir; and additive or synergistic effects with didanosine, zalcitabine, stavudine, and lamivudine **(Abstract 632).** The authors further noted abacavir synergy with zidovudine-lamivudine against zidovudine-susceptible and -resistant strains.

The resistance mutations in the RT gene selected in vitro by abacavir at residues 65, 74, 115, and 184 were noted in an isolate selected in vivo, as reported by Larnier and the Abacavir Investigative Team **(Abstract 686).** This genotype was associated with a threefold increase in the viral IC_{50}. Other substitutions were noted with greater losses of susceptibility to the drug. In a separate study by Mellors et al, more than 90% of HIV-1 isolates that were resistant to zidovudine, lamivudine, or zidovudine/lamivudine remained susceptible to abacavir in vitro **(Abstract 687);** however, the virologic responses in patients with these isolates were less encouraging. Resistant isolates with loss of susceptibility to additional nRTIs, however, were less likely to retain susceptibility to abacavir. In this and the prior study, baseline phenotypic resistance more than eightfold above the wild-type IC_{50} was predictive of a poor virologic response at 4 weeks of therapy.

Data for the triple-nRTI regimen abacavir/zidovudine/lamivudine were presented by Staszewski et al. Among antiretroviral-naive subjects who received this open-label combination, 60% had plasma HIV-1 RNA levels below 400 copies/mL and 48% had fewer than 50 copies/mL at 48 weeks, (the median baseline level was approximately 5 log) **(Abstract 658).** In a separate study also reporting antiretroviral effects of abacavir in combination with other antiretrovirals including nRTIs, 8 of 15 patients had plasma HIV-1 RNA levels <400 copies/mL at 48 weeks **(Abstract 659).**

Results from trials using abacavir in combination with protease inhibitors were also presented. Mellors et al reported data collected from the CNAA2004 Trial, which enrolled 80 antiretroviral-naive subjects (median plasma HIV-1 RNA levels and CD4+ cell counts of 4.74 log and 349/μL, respectively), who were randomized to receive abacavir combined with amprenavir, indinavir, ritonavir, saquinavir SGC, or nelfinavir **(Abstract 4).** After 16 weeks on therapy, between 50% and 85% of subjects had plasma HIV-1 RNA levels <400 copies/mL, and 40% to 70% had <50 copies/mL. Immunologic changes included approximate median absolute increases of 160 total CD4+ cells/μL, 20 naive CD4+ cells/μL, and 100 memory CD4+ cells/μL from baseline at week 16 **(Abstract 364).**

FTC.— Pottage et al presented data on the efficacy of the cytidine analogue, FTC (LB9). In vitro studies have shown that FTC is synergistic with zidovudine, stavudine, efavirenz, MKC-442, an NNRTI, indinavir, and nelfinavir. In this phase I/II clinical trial, patients with mean baseline HIV-1 RNA levels between 4.2 and 4.7 log received FTC at oral doses of either 25 mg or 200 mg bid. At the higher dose, plasma viremia was reduced by an average of 2.1 log after 14 days without significant adverse effects.

Nucleotide Analogue Reverse Transcriptase Inhibitors

Adefovir.— A study of adefovir-resistant (bis-pom-PMEA) isolates selected in vivo presented by Miller et al demonstrated K70E and T69D mutations in the RT gene **(Abstract 677).** The K70E genotype was associated with a 2- to 3-fold decrease in virus susceptibility to adefovir. This genotype showed 4.9-fold and a 2.7-fold decreases in PMEA and lamivudine susceptibility, respectively. The presence of the M184V mutation in high-level zidovudine/lamivudine-resistant isolates reversed PMEA resistance by 2.6 to 4.4-fold. The significance of this resistance mutational interaction is unclear.

PMPA.— Wainberg et al demonstrated that in vitro selection of PMPA resistance results in K65R mutation with a 3-fold increase in IC_{50} and moderate cross-resistance to zalcitabine, didanosine, lamivudine, and adefovir **(Abstracts 630,680).** HIV-1 strains resistant to zidovudine, didanosine, or multiple nRTIs (containing the Q151M gene complex) remained susceptible to PMPA. Similar to PMEA, isolates containing the lamivudine-associated M184V substitution had increased susceptibility to PMPA **(Abstracts 677,680).**

Deeks et al presented phase I/II bis-poc-PMPA dose-escalation data (LB8). The oral bioavailability of this drug is 41% when taken without food (reduced to 27% when taken with food) and it has a half-life of more than 17 hours. In initial clinical efficacy studies, plasma HIV-1 RNA levels returned to pretreatment values in all doses studied by day 60 of treatment in patients with a baseline CD4 cell count of 375 cells/μL and plasma HIV-1 RNA level of 4.5 log Significant adverse effects included elevations in transaminases and creatine kinase.

NNRTIs

Efavirenz (DMP 266)—. From two studies, DMP 266-005 and DMP 266-003, data were presented on efavirenz combined with two nRTIs or a protease inhibitor **(Abstracts 692,698).** When efavirenz at 600 mg/d, was taken with zidovudine and lamivudine by antiretroviral-naive individuals for 24 weeks, the mean plasma HIV-1 RNA level was diminished from a pretreatment level of 4.7 log by approximately 2 log. Ninety-percent of these patients had plasma viremia reduced to less than 400 copies/mL, and 67% to below 40 copies/mL **(Abstract 698).** In the DMP 266-003 study, Kahn, on behalf of DMP 266 Clinical Development Team, reported that efavirenz combined with indinavir in patients, the majority of whom (71%) had prior nRTI experience, resulted in a mean reduction in plasma HIV-1 RNA levels of 2.5 log (91% to below 400 copies/mL) and a mean increase in CD4+ cell count of 267/μL after 60 weeks of therapy **(Abstract 692).** Nausea, headache, and fatigue were the most commonly encountered adverse effects.

Efavirenz resistance was most commonly associated with a K103N substitution (19-fold decreased susceptibility to the drug) in both in vivo and in vitro studies **(Abstracts 702,703).** This mutation mediates cross-resistance to nevirapine (40-fold decrease); delavirdine (28-fold decrease); and loviride (7-fold decrease). In drug susceptibility testing, viral isolates with the NNRTI-associated resistance mutation, Y181C, and the delavirdine-associated resistance mutation P236L retained susceptibility to efavirenz **(Abstract 702).**

Protease Inhibitors

Amprenavir(141W94;VX-478).— Studies were presented that employed amprenavir in monotherapy or in combination with nRTIs. Murphy et al reported the results of ACTG 347, which analyzed amprenavir alone or combined with zidovudine/lamivudine in protease inhibitor- and lamivudine-inexperienced patients. The median CD4+ cell count and plasma HIV-1 RNA level was of 305/μL and 37,889 copies/mL, respectively **(Abstract 512).** The amprenavir monotherapy arm was discontinued after a median of 88 days due to 9 cases of early virologic failure. At 24 weeks, 63% of patients in the 3-drug arm had plasma HIV-1 RNA levels <400 copies/mL.

The efficacy of combination amprenavir/abacavir in antiretroviral-naive patients (median baseline plasma HIV-1 RNA of 4.38 log copies/mL and CD4+ cell count of 619/μL) was reported by Bart et al **(Abstract 365).** After 24 weeks of therapy, 9 of 11 patients had plasma HIV-1 RNA values below 50 copies/mL.

A four-drug combination of abacavir/zidovudine/lamivudine/amprenavir was assessed by Kost et al in acutely (<90 days since exposure) and chronically HIV-1-infected, protease inhibitor- and lamivudine-naive subjects **(Abstract 363)**. With mean baseline plasma HIV-1 RNA levels of 192,641 and 57,174 copies/mL, at week 20, 4 of 7 and 8 of 9 subjects lowered these levels to below 100 copies/mL, respectively. In an analysis of non-plasma viral load effects in a compartment other than plasma, CSF HIV-1 RNA levels were reduced from a mean baseline of 1,644 copies/mL and 8,093 copies/mL by 1.22 log by week 8 in the acutely-infected and chronically-infected individuals, respectively. Immunologic effects at week 12 were notable for increases in CD4+CD62L+RA+ naive cells of 106 and 29/μL, respectively.

The preliminary efficacy of amprenavir-containing dual protease inhibitor therapy was illustrated by the PROA2001 study that enrolled protease inhibitor-naive patients treated with amprenavir combined with a second protease inhibitor including indinavir, nelfinavir, and saquinavir SGC **(Abstract 6)**. Baseline median logplasma HIV-1 RNA levels ranging from 4.45 to 5.14 were reduced by 2.53 and 3.18 log copies/mL.

The genotypic characterization of HIV-1 isolates from patients in whom amprenavir therapy was failing in the ACTG 347 study was presented by DePasquale et al **(Abstract 406a).** In this analysis, two classes of amprenavir monotherapy mutations were described: (1) I50V-containing isolates with associated companion mutations within the protease coding region or an associated gag cleavage site mutation (I to F substitution at the P1/P6 gag cleavage site); and (2) or alternatively, isolates with protease coding region mutations other than I50V also accompanied by gag cleavage site changes. Failure of amprenavir/zidovudine/ lamivudine combination therapy was related to the presence of the M184V lamivudine resistance associated mutation in the RT gene; substitutions in the protease coding region residues 10, 20, 46, 50, 54, and 82; or gag cleavage site changes.

PNU-140690.— Initial safety and pharmacokinetic data of PNU-140690, a potent, non-peptidic protease inhibitor of the dihydropyrone class with activity versus ritonavir resistant isolates were presented in two posters. In healthy volunteers, PNU-140690 systemic exposure was increased by two-fold with high-fat food and reduced by co-administration with antacids by 33% **(Abstract 649).** The major adverse effects were nausea, diarrhea, and abdominal cramps **(Abstract 648).**

Other Agents

Pre-clinical data were presented on a number of antiretroviral compounds including the reverse transcriptase inhibitors dd4FC, which lacks cross-resistance to the approved nRTIs **(Abstract 629);** the cyclopropane, QYL-685, which selects for a M184I mutation in vitro; BCH-10652, a 4'-thio heterosubstituted nRTI with low plasma protein binding and activity against viral strains resistant to lamivudine, zalcitabine, zidovudine, and PMEA **(Abstract 628);** +/- calanolide A, an NNRTI; and PD178390, a non-peptidic dihydropyrone protease inhibitor, which retains activity against protease inhibitor resistant HIV-1 strains with substitutions at residues G48, M46, V82, V84, and D30 **(Abstracts 637,638).**

Treatment Failure and Salvage Therapies

Predictors of Response to Initial Protease Inhibitor Therapy

Demeter, on behalf of the ACTG 320 Study Team, presented an examination of predictors of virologic response **(Abstract 509).** The subjects were zidovudine-experienced with CD4+ cell counts 200/μL or below and were randomized to indinavir/zidovudine/lamivudine or zidovudine/lamivudine. The strongest independent predictor of week 24 and 40 viral suppression was the absolute week 4 to 8 plasma HIV-1 RNA concentration. In an analysis of predictors of immunologic response to the three-drug, protease inhibitor regimen used in ACTG 315 (ie, ritonavir/zidovudine/lamivudine), the return of a delayed type hypersensitivity reaction did not correlate with the amplitude of plasma HIV-1 RNA suppression and only weakly correlated with the degree of CD4+ cell count increase (LB14).

Antiretroviral Resistance Interactions

In part, a rational formulation of a potential salvage regimen is dependent upon a consideration of resistance and cross-resistance patterns of the antiretrovirals which comprise the failed regimen. Accordingly, there were several reports of genotypic and phenotypic resistance patterns to these agents alone and in combination with other antiretrovirals.

Reverse transcriptase inhibitor resistance.— Several studies examined the effects of the preceding use of specific drugs on the likelihood of developing subsequent resistance in vitro to other nRTIs. Miller et al found that the number of previously used nRTIs correlated with resistance to zalcitabine, lamivudine, and stavudine; previous use of lamivudine was associated with increased risk of stavudine resistance; and previous use of didanosine or stavudine was associated with increased risk for lamivudine resistance **(Abstract 674).** In an investigation of potential mechanisms by which prior nRTI use could affect the subsequent efficacy of a second drug, Sommadossi et al found that the intracellular phosphorylation to the active nucleoside triphosphate form significantly correlated with antiretroviral response to stavudine and lamivudine **(Abstract 362).** Moreover, long-term zidovudine treatment resulted in diminished stavudine and lamivudine phosphorylation and preceding zidovudine/stavudine was associated with decreased stavudine phosphorylation for weeks after the discontinuation of zidovudine. These two observations may partially explain the diminished efficacy of stavudine following zidovudine therapy and the zidovudine/stavudine antagonism.

Palmer et al examined the cross-resistance patterns of two multidrug resistant viral strains, one containing four reverse transcriptase inhibitor resistance mutations (75I, 77L, 116Y, 151M), and the other containing seven reverse transcriptase inhibitor resistance mutations (41L, 43N, 67N, 118I, 184V, 210W, and 215Y) **(Abstract 405).** The former conferred high-level phenotypic resistance to multiple inhibitors including abacavir, F-ddA, PFA foscarnet, zidovudine, stavudine, didanosine, and lamivudine and partial susceptibility to PMEA and PMPA, and the latter conferred substantial resistance to abacavir, F-ddA, zidovudine, stavudine, didanosine, and lamivudine.

Protease inhibitor resistance.— Presentations of cross-resistance patterns among protease inhibitors included a study of HIV-1 clinical isolates by Hertogs et al, which defined resistance characteristics among indinavir, saquinavir, ritonavir, and nelfinavir **(Abstract 395).** General findings included the observation that in 77% to 95% of viral isolates with tenfold resistance to one of these four protease inhibitors, there was an association with at least a fourfold rise to the other three agents; in 62% to 79% this cross-resistance was at least tenfold. Mutations in the HIV-1 gag protease cleavage sites were also seen, most commonly an A to V substitution at the p7/p1 site, and a majority of resistant genotypes included multiple mutations (most

commonly 5). In a separate study, the appearance of these gag cleavage site substitutions did not correlate with the number of protease coding region mutations or the duration of protease inhibitor therapy **(Abstract 402).**

Salvage Therapy Regimens

Dual Protease Inhibitor Combinations

Ritonavir/saquinavir:—Tebas et al reported the use of ritonavir/saquinavir/stavudine/lamivudine following nelfinavir failure in either the Agouron 506 or 511 trials **(Abstract 510).** While 9 of 10 patients with limited antiretroviral therapy prior to nelfinavir responded to this salvage regimen, only 43% (3 of 7) with more extensive antiretroviral experience had reductions in plasma HIV-1 RNA levels to below 500 copies/mL **(Abstract 510).**

Duncombe et al reported the use of ritonavir/saquinavir combined with zidovudine/lamivudine or stavudine/lamivudine in 58 patients, 66% of whom were protease inhibitor-experienced (50% saquinavir- and 14% ritonavir-experienced) **(Abstract 390).** From a median plasma HIV-1 RNA of 4.49 log and a CD4 cell count of 191/μL, HIV-1 plasma viremia was decreased by a median of 1.98 log; 49% of subjects to below 400 copies/mL. In this study, neither protease inhibitor nor nRTI pretreatment correlated with virologic response. A third ritonavir/saquinavir/ dual nRTI salvage study presented by Cassano et al enrolled 43 patients in whom single protease inhibitor-based triple drug therapy had failed **(Abstract 423).** After 9 months of follow-up, patients in whom saquinavir and ritonavir or indinavir had failed reduced their plasma HIV-1 RNA levels by 1 and 1.5 log copies/mL, respectively. Respective CD4+ cell increases were 100 and 50/μL.

Response to ritonavir/saquinavir salvage therapy appears to be dependent on several factors. In the study by Tebas et al, higher plasma HIV-1 RNA levels at the time of the regimen change were associated with treatment failure. The presence of specific resistance genotypes (eg, the L90M mutation in the protease gene) was not predictive of treatment failure, although there was a trend toward an association with lamivudine resistance. In contrast, a virologic analysis of ACTG 333 showed that an HIV-1 protease residue 10 mutation predicted subsequent failure to indinavir in patients previously treated with saquinavir hard gelatin capsule **(Abstract 511).**

A related retrospective analysis presented by Gallant et al suggested that treatment with ritonavir/saquinavir and two different nRTIs, after a loss of viral suppression with either indinavir or nelfinavir, was more likely to succeed if the new regimen was initiated when plasma viral loads were relatively low **(Abstract 427).** Responding patients (ie, subjects with plasma HIV-1 RNA levels <400 copies/mL after 16 weeks of therapy) had a mean plasma viral level of 12,562 copies/mL compared with 33,367 copies/mL in non-responders. The importance of changing the accompanying nRTI regimen concurrently with the protease inhibitor was demonstrated in a study presented by Rozenbaum et al in which the nRTI switch correlated with a 3.5-fold greater likelihood of achieving a plasma HIV-1 RNA level below 500 copies/mL after 10 months of therapy **(Abstract 420).** Bodsworth et al similarly demonstrated a virologic benefit to changing or adding to the accompanying nRTI regimen **(Abstract 396).**

Protease Inhibitor/NNRTI Combinations

Indinavir/nevirapine combinations.— Several reports of using indinavir/nevirapine in antiretroviral-experienced populations were presented. In patients with a median CD4+ cell count and plasma HIV-1 RNA level of 30/μL and 5.16 log copies/mL, respectively, who had failed or were intolerant to nRTI therapy, a regimen consisting of indinavir/nevirapine/lamivudine diminished the plasma HIV-1 RNA level to below 400 and to below 40 copies/mL in 45% and 32% of the patients, respectively, after 1 year of therapy **(Abstract 429a).** Murphy et al reported on 36 subjects who had been rolled over from the amprenavir monotherapy arm of ACTG 347. A combination of nevirapine/indinavir/ stavudine/lamivudine reduced plasma viremia to below 400 copies/mL in 32 of 36 of these individuals **(Abstract 512).** A third study by Lawrence et al used indinavir/nevirapine and two nRTIs for patients with virologic failure of saquinavir and/or nelfinavir **(Abstract 422).** At enrollment, the median plasma HIV-1 RNA level was 4.22 log. The combination of indinavir/nevirapine yielded a modest 0.9 log reduction in plasma HIV-1 RNA level in this patient population.

Nelfinavir/nevirapine-based combinations.— Similar to the modest and unsustained virologic responses seen with indinavir/nevirapine salvage therapy described above, Gerard et al reported that nelfinavir/nevirapine combined with foscarnet for the initial three weeks, while resulting in a 2.64 log reduction in plasma HIV-1 RNA levels at week 3, was unable to maintain viral suppression past week 8 in heavily pretreated patients **(Abstract 424).** A second small study using a 6-drug regimen consisting of nelfinavir/nevirapine/saquinavir/lamivudine/stavudine/didanosine in 12 patients with extensive protease and reverse transcriptase inhibitor experience, but who were nelfinavir/nevirapine-naive, preliminarily reported that at 12 weeks, 9 of the 12 patients decreased their plasma HIV-1 RNA

levels to below 400 copies/mL from a median of 64,000 copies/mL **(Abstract 426).** The durability, tolerability, and safety of regimens like this need to be determined.

ANTIRETROVIRAL STRATEGIES

Induction-Maintenance

Two studies were reported that demonstrated the failure of 3- to 6-month induction periods combined with a dual nRTI protease inhibitor/nRTI, or protease inhibitor monotherapy maintenance regimen. ACTG 343, presented by Havlir et al, was a trial that employed indinavir/zidovudine/lamivudine induction followed by randomization to one of three maintenance regimens if plasma HIV-1 RNA levels were reduced to below 200 copies/mL at weeks 16, 20, and 24: the same 3-drug regimen; indinavir monotherapy; or zidovudine/lamivudine (LB16). Subjects had no protease inhibitor or lamivudine experience prior to induction therapy and had median baseline CD4+ cell counts between 437 and 463 cells/μL and plasma HIV-1 RNA levels between 17,000 and 22,000 copies/mL at the time of randomization to the maintenance regimens. Using the primary study endpoint of virologic failure defined by two consecutive plasma HIV-1 RNA levels greater than 200 copies/mL, the 3-drug maintenance regimen was substantially more effective than either indinavir monotherapy or the dual nRTI arms (3%, 23%, and 23%, respectively). Predictors of virologic failure in the zidovudine-lamivudine arm included more than 6 months of prior zidovudine use and the presence of the zidovudine-associated 215 resistance mutation. Indinavir monotherapy maintenance versus the 3-drug regimen carried a sevenfold increased relative risk for failure. A greater increase in CD4+ cell count in response to induction was associated with a 1.4-fold higher relative per 100 CD4+ cell rise risk, possibly reflective of a larger HIV-infectable cellular pool in the setting of inadequate viral suppression on maintenance therapy.

In the TRILEGE (ANRS 072) trial, the same induction regimen was used in antiretroviral naive patients with a mean baseline CD4+ cell count and plasma HIV-1 RNA level of 363/μL and 4.48 log, respectively (LB15). After 3 months of induction, subjects with plasma viral levels below 500 copies/mL were randomized to either continued indinavir/zidovudine/lamivudine, zidovudine/lamivudine, or indinavir/zidovudine maintenance. The primary study endpoint was virologic failure as defined by a resumption in measurable (>500 copies/mL) HIV-1 viremia. Six months after randomization, patients who were maintained on 3-drugs were significantly less likely to fail relative to either dual drug arms (10%, 38%, and 24%, respectively). Further validation of induction-maintenance strategies will be dependent upon studies utilizing more prolonged durations of induction and/or more potent maintenance regimens.

Conclusions

Studies reported at the 5th Conference on Retroviruses and Opportunistic Infections taken together form the most comprehensive summary to date of the state-of-the-art in antiretroviral chemotherapy. The principle of combining maximal regimen potency with close virologic monitoring was reinforced and the increasing number of potentially effective combinations was highlighted. Simultaneously, the shortcomings of the current therapeutic armamentarium were detailed and the spotlight placed on areas in which future research efforts must be focused. Most importantly, the overall message was one of continued progress in the field and cautious optimism for the future. ■

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