New Nucleoside Analogues and Nonnucleoside RTIs

New nucleoside reverse transcriptase inhibitors (RTIs) and nonnucleoside RTIs (NNRTIs) were discussed at the New York meeting by Gerald H. Friedland, MD, from Yale New Haven Hospital and Yale University School of Medicine, New Haven, Connecticut, and at the Chicago meeting by John C. Pottage, Jr, MD, from Rush Medical College, Chicago, Illinois.

Despite enthusiasm over nonnucleoside analogue anti-HIV agents, the nucleoside RTIs remain the mainstay of antiretroviral therapy for the foreseeable future. There are several reasons for the continued interest and reliance on these agents. Available nucleoside RTIs have presently been limited in application because of dose-related toxicities, although further development of agents in this class may result in agents that retain the antiviral activity with less toxicity. In addition, some of the newer agents have characteristics that render them attractive as alternative treatment options. Finally, with the advent of the era of combination therapy, the nucleosides are likely to be utilized as the core of combination regimens as the characteristics of the newer classes of agents (eg, NNRTIs and protease inhibitors) are more clearly defined. Thus, it will remain important to know which of the nucleosides are most beneficial, least toxic, and most durable in effects in terms of both combination therapy and monotherapy.

Stavudine

Stavudine (d4T) was the fourth nucleoside analogue RTI to become available in the United States. Approval, based on data from studies of more than 11,000 largely zidovudine-experienced patients, indicated a beneficial effect on virologic and immunologic markers. Stavudine is characterized by rapid oral absorption, high absolute bioavailability that is unaffected by pH, absence of significant accumulation, and significant penetration into the cerebrospinal fluid (20% to 70%). Dose-ranging studies showed dose-proportional antiviral effects and dose-related toxicity, with the primary toxicity being peripheral neuropathy. These studies suggested an appropriate clinical dosage of 0.5 to 1.0 mg/kg, although investigation of higher dosages continues on the basis of postlicensure experiences that have indicated that toxicities, particularly peripheral neuropathy, are not so frequent as suggested in the early studies.

Several early-phase studies of stavudine indicated that treatment at doses of 0.5 to 1 mg/kg/d resulted in improvements in CD4+ cell count and HIV p24 antigen level that were sustained for 24 to 36 weeks, with a decrease in peripheral blood mononuclear cell (PBMC) viral titer of 0.5 to 2 logs being observed in the small number of cases. More recently, stavudine 40 mg bid was compared with zidovudine 200 mg tid in a double-blind, randomized trial sponsored by Bristol-Myers Squibb (BMS 019) in patients with CD4+ cell counts of 50 to 500/μL who had received more than 6 months of prior zidovudine. Among the 822 patients enrolled, the median CD4+ cell count was 235/μL and the mean duration of prior zidovudine treatment was 88 weeks.

Preliminary findings of the study were discussed by Dr Pottage at the Chicago meeting. A comparison of CD4+ cell count responses in the first 359 patients enrolled in the study showed that the difference between changes in CD4+ cell count significantly favored stavudine treatment over continued zidovudine over the course of the study. As stated by Dr Pottage, treatment failure in the protocol was defined as a 50% decline in CD4+ cell count, occurrence of an AIDS-defining event, or death. Data on the entire patient population, with follow-up of up to more than 2.5 years, revealed a number of findings favoring the switch to stavudine. Stavudine patients remained on drug for an average of approximately 79 weeks whereas zidovudine recipients remained on drug for approximately 50 to 55 weeks—a significant difference—with the primary reason for premature zidovudine discontinuation being the occurrence of clinical events.

In BMS 019, the switch to stavudine was associated with a significantly decreased rate of treatment failure compared with continued zidovudine in patients with more than 6 months prior zidovudine treatment; no significant difference in mortality was observed.

The rate of treatment failure (CD4+ cell count decline >50% of baseline and progression to clinical endpoints) among stavudine recipients was significantly lower than that among patients receiving continued zidovudine. Analysis of clinical progression—that is, reaching clinical endpoints alone—also showed that stavudine treatment was significantly superior to continued zidovudine, although the difference between the two groups was reduced somewhat compared with that for overall failure rate. Although the mortality rates in the two groups were not significantly different, a difference favoring stavudine treatment approached significance ($P = 0.07$).

Data from the entire patient population showed that peripheral neuropathy occurred significantly more frequently among stavudine recipients, being observed in 18 (4%) of 405 zidovudine patients and 56 (13%) of 417 stavudine patients ($P = 0.0005$). According to Dr Pottage, stavudine was better tolerated with regard to other adverse events, including gastrointestinal events and hematologic toxicity. According to data on the first 389 patients enrolled, presented by Dr Friedland, there were significant differences favoring stavudine in terms of hematologic abnormalities, with incidence of other laboratory abnormalities in the two groups being similar: hemoglobin levels of <11 g/dL developed in 5% of stavudine patients and 11% of zidovudine patients ($P = 0.01$); white blood cell counts of less than 4000/μL developed in 62% and 76%, respectively ($P = 0.001$); and polymorphonuclear cell counts of less than 1500/μL developed in 29% and 48%, respectively ($P = 0.0001$). The use of a standard-

TABLE 1. RISK FACTORS FOR PERIPHERAL NEUROPATHY IN THE STAVUDINE PARALLEL TRACK PROGRAM

Variable	Relative risk	95% CI	*P* value
Dose, 40 mg:20 mg	1.39	1.26, 1.53	0.0001
Prior neuropathy	1.68	1.52, 1.86	0.0001
CD4+ count, baseline*	1.03	1.01, 1.04	0.0001
Hemoglobin ≤11 g/dL	1.23	1.10, 1.38	0.0005
Male	1.33	1.04, 1.71	0.02
Karnofsky index <80	1.13	1.02, 1.27	0.03

*Square root scale—eg, 25 cells vs 16 cells. CI indicates confidence interval.

ized instrument for assessing quality of life showed a number of significant differences on health and function measures, suggesting that stavudine patients felt substantially better than patients remaining on zidovudine. Significant differences in changes from baseline measurements were observed for general health, mental health, physical function, emotional role, physical role, social function, and vitality indices.

Dr Friedland presented data on risk factors for peripheral neuropathy from the stavudine parallel track program, in which patients with no therapeutic alternatives—ie, patients with very advanced illness who had extensive prior treatment with the other available nucleoside analogues—had been randomized to stavudine 20 mg bid or 40 mg bid. Among the factors associated with a significantly increased risk of neuropathy were the higher stavudine dosage (40 mg bid) and male gender (see Table 1). Other significant predictors included factors associated with more advanced disease—ie, prior neuropathy, lower hemoglobin level, lower CD4+ cell count, and lower Karnofsky score.

Dr Friedland presented findings of a retrospective analysis of response to stavudine in zidovudine-naive patients from the stavudine data base, noting the limitations and potential hazards of such analyses. As noted, the vast majority of patients receiving stavudine in early studies were zidovudine experienced. A total of 75 zidovudine-naive patients were identified from phase I/II studies. As shown in Figure 1 (top), CD4+ cell response among zidovudine-naive patients was considerably better than that in zidovudine-experienced patients, with the former exhibiting a peak mean increase of 110/μL by week 12 and maintaining counts above baseline for more than 48 weeks. Responses in the zidovudine-naive stavudine recipients were also compared with those in previously zidovudine-naive zidovudine recipients in ACTG 116A with matched initial CD4+ cell counts 300/μL or lower. Figure 1 (bottom) shows the comparison of 31 patients receiving stavudine as initial therapy and 93 patients with matched CD4+ cell counts who received zidovudine as initial therapy. The mean maximum increases were 94/μL in the former and 53/μL in the latter, with increases above baseline being maintained for greater than 48 weeks in the stavudine patients and for 30 weeks in the zidovudine patients. As related by Dr Friedland, although these analyses are open to serious bias, they have at least suggested that a head-to-head comparison of the two agents is warranted. Such a comparison is planned in ACTG 298, in which patients with CD4+ cell counts of 200 to 600/μL who have not received prior antiretroviral therapy will receive stavudine, zidovudine, or the combination of the two. Outcome measures will include viral load and CD4+ cell count responses, development of resistance, and the possibility of pharmacologic interaction.

Some early in vitro pharmacologic data suggested potential pharmacologic antagonism between stavudine and zidovudine through competition for intracellular phosphorylating enzymes. However, more recent data from a number of laboratories evaluating in vitro antiretroviral activity of the combination indicate not only absence of antiviral antagonism but an additive or synergistic effect of the two under most conditions. Data from

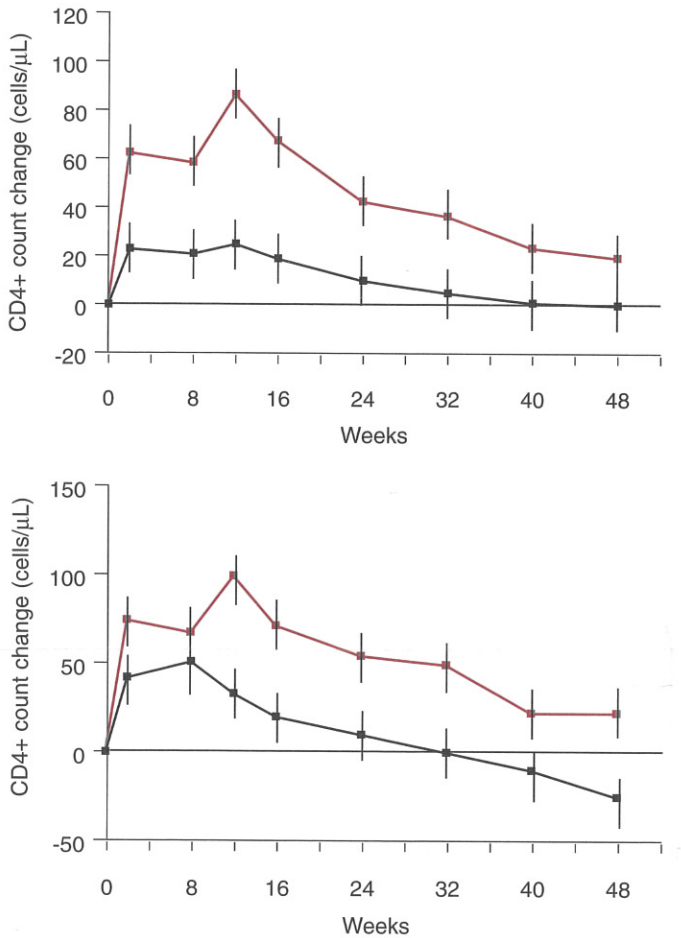

Figure 1. Top. Mean CD4+ cell count change during stavudine treatment among a small population of zidovudine-naive patients (red line) and the larger population of zidovudine-experienced patients (black line) in the stavudine study data base (BMS 002, 003, and 006). Bottom. Mean change in CD4+ cell count among 31 zidovudine-naive patients receiving stavudine (red line) in BMS 002, 003, and 006 and 93 zidovudine-naive zidovudine recipients (black line) in ACTG 116A matched for CD4+ cell counts ≤300/μL.

the combination arm of this trial will help to determine if the combination will be of clinical utility. A trial comparing the two agents alone and in combination in symptomatic pediatric patients with little or no prior treatment is currently underway (ACTG 240).

One potential benefit of stavudine is that resistance to the agent, although apparently inevitable and demonstrably inducible in serial passage in culture, appears to develop quite slowly despite the relatively strong antiviral activity of the agent. Currently, there are few data on development of resistance in the clinical setting, and more systematic study is warranted. Although, as with other nucleoside analogues examined, there is a time-limited effect of stavudine on immunologic and virologic measures, decreased susceptibility of virus appears to be infrequent and of small magnitude.

As shown in Table 2, study of pretreatment and posttreatment isolates from 11 patients receiving 18 months of treatment in BMS 006 showed decreased susceptibility in two of the posttreatment isolates, with sensitivities of these isolates nevertheless remaining within the range of therapeutically achievable concentrations. No genetic basis for the decreased sensitivity was determined, suggesting that the susceptibility change in PBMC was an artifact, resulting from the rapid growth characteristics of the later virus in PBMC. An interesting finding was that five of the patients yielded zidovudine-resistant virus after 18 months, with no stavudine cross-resistance being observed; the authors of the study concluded that off-study administration of zidovudine had probably occurred. Another analysis of isolates from eight patients from BMS 009 after 12 months of treatment showed decreased sensitivity in one posttreatment isolate. The mean stavudine 50% effective dose (ED_{50}) for the remaining isolates was unchanged after 52 weeks of treatment. As related by Dr Pottage, future ACTG studies of stavudine include ACTG 290, which evaluates stavudine vs didanosine vs zidovudine plus stavudine or didanosine in patients with CD4+ cell counts of 300 to 600/μL and greater than 12 weeks of prior zidovudine and ACTG 298, which evaluates stavudine vs zidovudine vs the combination of the two in patients with CD4+ cell counts of 300 to 600/μL and no prior nucleoside analogue RTI therapy. Dr Friedland identified a number of questions regarding the role of stavudine in therapy (see Table 3).

Although apparently inevitable and demonstrably inducible in culture, resistance to stavudine appears to develop slowly despite the relatively strong antiviral activity of the agent.

Table 2. Stavudine Resistance

- Limited in vivo information
- 11 pairs from BMS 006 treated for 18 months*
  - 9/11 posttreatment isolates unchanged
  - 2/11 posttreatment isolates had decreased sensitivity
  - Genetic basis of decreased sensitivity not determined
  - 5/11 developed zidovudine resistance
- 8 pairs from BMS 009 treated for 12 months
  - 1/8 posttreatment isolates had decreased ED_{50} (0.2 to 1.2 mM)

*Lin et al. *J Infect Dis* 1994.

Table 3. Stavudine: Unanswered Questions

- Will existing studies demonstrate clinical benefit for stavudine?
- What is the role of stavudine in early therapy?
- Will the relative lack of development of resistance hold up over time?
- Can stavudine be combined with zidovudine and other antiretroviral agents with similar toxicities?
- What is the role of stavudine in combination therapy?
- What combinations offer the most sustained benefits and least toxicity?

Lamivudine

Currently there is great interest in the therapeutic potential of lamivudine (3TC). Preclinical findings indicate that the agent has activity against both HIV-1 and HIV-2, including zidovudine-resistant strains, and exhibits no inhibition of hematopoietic progenitor cells. In vitro, the agent is less active than zidovudine and zalcitabine and more active than didanosine, and exhibits synergy with zidovudine. Lamivudine is markedly less toxic to host cells than either zidovudine or zalcitabine, exhibiting a favorable cytotoxicity profile comparable to that of didanosine. Phase I studies demonstrated that the drug is rapidly absorbed after oral administration, with high absolute bioavailability and no effect of food on overall absorption. It exhibits a serum half-life of 2 to 4 hours and a protracted intracellular half-life of approximately 12 hours. Elimination is primarily renal in the form of unchanged drug (approximately 70%). High-level resistance to lamivudine develops rapidly in vitro in association with a single amino acid substitution at RT codon 184. Although most information on lamivudine resistance has come from in vitro studies, resistance emerges uniformly within weeks in the clinical setting. The full implications of resistance in vivo have yet to be elucidated. Observations in this area thus far include: (1) maintenance of virologic response despite presence of resistance, (2) an interaction with zidovudine in the setting of combination therapy whereby zidovudine resistance may be delayed and whereby zidovudine-resistant virus may revert to susceptibility, and (3) continued susceptibility of isolates with the codon 184 mutation to zidovudine and stavudine in the absence of other RT codon changes.

Dr Friedland summarized initial findings in two North American (NUCA) and two European (NUCB) pivotal lamivudine phase II/III trials in adults; each program included one study

in zidovudine-naive patients and one in zidovudine-experienced patients. In NUCA 3001, 364 patients with 4 weeks or less prior zidovudine and CD4+ cell counts of 200 to 500/μL were randomized to double-blind treatment with lamivudine 150 or 300 mg bid plus zidovudine 200 mg tid or monotherapy with lamivudine 300 mg bid or zidovudine 200 mg bid. Data on mean actual changes in CD4+ cell count showed that response in the combination treatment arms was markedly better than that in the monotherapy arms, with counts remaining above baseline levels for at least 52 weeks in the former.

In NUCB 3001, 129 patients with CD4+ cell counts of 100 to 400/μL and 4 weeks or less of prior zidovudine were randomized to double-blind treatment with zidovudine 200 mg tid or zidovudine plus lamivudine 300 mg bid. After 24 weeks, all patients receiving zidovudine alone had lamivudine added to treatment. CD4+ cell responses were dramatically better in the combination treatment group during double-blind treatment, with cell counts returning to baseline in the zidovudine monotherapy group by week 24. With the addition of lamivudine at week 24, cell counts in the original monotherapy group then increased to levels comparable to those in the patients who were originally randomized to and continued to receive combination therapy. Counts in both groups remained above baseline after 56 weeks. Identical effects on viral load were observed in the subgroup of patients assessed by plasma HIV RNA polymerase chain reaction (PCR) assay. The combination treatment patients exhibited a median 1.5 log drop in plasma HIV RNA level—a decrease markedly greater than that in the zidovudine monotherapy patients. When lamivudine was added to monotherapy at week 24, viral load persistently decreased until it was at a level similar to that in the original combination treatment patients by week 48, with both groups exhibiting a level approximately 1 log lower than baseline levels at that time. Dr Friedland noted that the decrease in viral load with combination treatment was impressive compared with decreases reported with other nucleoside analogue combinations.

In NUCB 3002, 223 patients with CD4+ cell counts of 100 to 400/μL who had received ≥24 weeks of prior zidovudine were randomized to double-blind treatment with zidovudine 200 mg tid alone or in combination with lamivudine 150 mg or 300 mg bid. After 24 weeks, open-label lamivudine was added in all zidovudine monotherapy patients. CD4+ cell counts rapidly dropped below baseline in the zidovudine monotherapy patients and remained above baseline for the 24 weeks of double-blind treatment in both combination treatment groups. With the addition of lamivudine in the initial zidovudine monotherapy group at 24 weeks, the CD4+ cell count in this group increased to above baseline levels and remained above baseline at week 48.

In NUCA 3002, 254 patients with CD4+ cell counts of 100 to 300/μL and ≥24 weeks of prior zidovudine treatment were randomized to double-blind treatment with zidovudine 200 mg tid plus lamivudine 150 mg or 300 mg bid or zalcitabine 0.75 mg tid. Mean actual changes in CD4+ cell count were greater in the two lamivudine combination arms than in the zidovudine plus zalcitabine arm, with counts remaining above baseline levels for at least 52 weeks in the former groups and remaining at or below baseline in the zidovidine plus zalcitabine group.

As an example of the type of safety data that are emerging from these trials, Dr Friedland presented frequencies of drug-related clinical adverse events in patients in study NUCB 3002, stating that the very minimal toxicity that has thus far been associated with lamivudine has consisted primarily of headache and nausea. A similar proportion of patients in the zidovudine monotherapy group (55%) and the two combination groups (55% and 56%) reported no drug-related events.

Clinical outcome data from lamivudine studies are not yet available and are awaited with considerable interest. Data on the use of the agent in combination with agents other than zidovudine currently are lacking. However, a number of trials of such combinations are in progress or design.

NNRTIs

The NNRTIs are a structurally diverse but functionally similar group of compounds that include TIBO derivatives, BHAP compounds (eg, atevirdine, delavirdine), nevirapine, and pyridinone derivatives (eg, L-697,661), all of which work by binding directly to RT. The agents are potent inhibitors of HIV-1, including zidovudine-resistant isolates. Because they rapidly select for resistant virus in vitro and in vivo, combination therapy is the primary role envisioned for these compounds. However, study of nevirapine monotherapy has shown that some patients have a persistent virologic and immunologic response to the agent despite the development of mutations conferring high-level resistance, suggesting that the susceptibility level of resistant virus can be exceeded in some cases. The agents that have been clinically investigated have been generally well tolerated. The characteristic primary adverse reaction is rash, which typically occurs early during treatment and can be treated through.

As noted by Dr Friedland, the use of nevirapine and zidovudine in combination does not prevent the emergence of nevirapine resistance. Data on nevirapine resistance mutations in isolates from patients receiving monotherapy in ACTG 164 and combination therapy in ACTG 168 indicate that although resistance is not prevented, the distribution of mutations at the RT

TABLE 4. EFFECT OF CONCOMITANT THERAPY WITH ZIDOVUDINE ON NEVIRAPINE RESISTANCE MUTATIONS

	Percent of patients with isolates developing mutations at indicated RT residue					
	103	106	108	181	188	190
Nevirapine monotherapy, ACTG 164; n=24	33	0	8	79	8	17
Nevirapine + zidovudine, ACTG 168; n=14	57	14	0	0	50	50

Adapted from Richman DD et al. *J Virol* 1994.

codons implicated in resistance is different under combination therapy (Table 4); for example, whereas the majority of monotherapy patients developed the characteristic codon 181 nevirapine resistance mutation, this mutation was not observed in isolates from the combination patients, with mutations at other codons being observed with increased frequency. These findings at once point out that the virus has an array of evolutionary options available to avoid susceptibility and that there may be an inherent constraint on the evolutionary pathways that can be taken. Exploitation of the latter feature in the clinical setting remains a matter for further study.

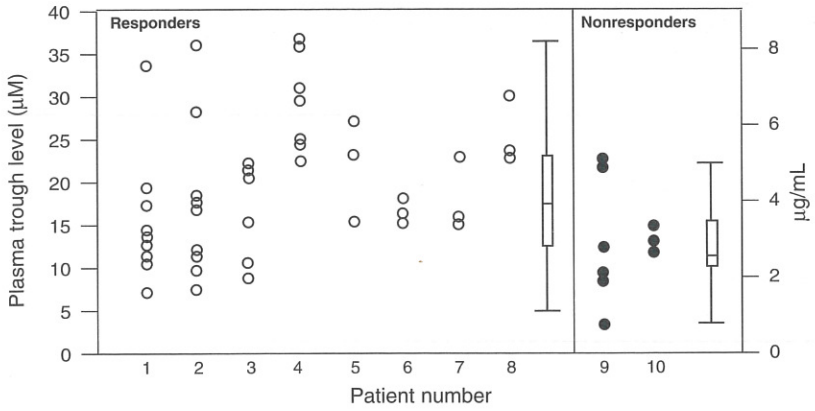

Figure 3. Plasma trough nevirapine levels in patients with (responders) or without (nonresponders) persistent surrogate marker response to nevirapine 400 mg/d. Adapted from Havlir D et al. J Infect Dis *1995.*

Another provocative phenomenon observed with nevirapine therapy is that, as noted above, a significant proportion of patients have a sustained antiviral response to higher-dose nevirapine despite the development of resistance. As presented by Dr Pottage, Figure 2 shows changes in HIV p24 antigen levels in patients receiving nevirapine 200 mg/d or 400 mg/d; as can be seen, the decrease among patients receiving the higher dosage was persistent, indicating ongoing antiviral effect. As presented by Dr Friedland, analysis of nevirapine plasma levels in patients receiving 400 mg/d who exhibited persistent response vs those who did not exhibit such response showed that trough levels were significantly greater in responding patients (Figure 3), suggesting that the susceptibility level of resistant virus can be overcome if sufficient blood-drug levels can be maintained; it is not yet known if it can be predicted which patients are likely to respond to nevirapine in this manner. It may be the case that similar phenomena occur with other antiretroviral agents.

Preliminary findings of ACTG 241, a phase II study of triple combination therapy including nevirapine vs double combination treatment, indicate superiority of the triple regimen with regard to

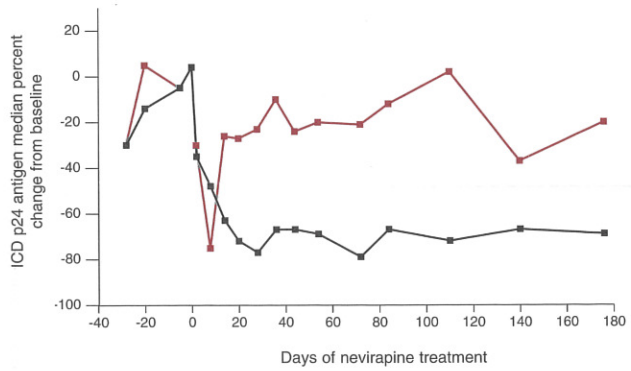

Figure 2. Median percentage change in immune complex dissociated (ICD) HIV p24 antigen in patients receiving nevirapine 200 mg/d (red line) or 400 mg/d (black line). Adapted from Havlir D et al. J Infect Dis *1995.*

virologic and immunologic markers. No clinical differences were observed, although the ability to detect a difference may have been beyond the scope of the study. In this study, patients with a CD4+ cell count <350/μL and ≥6 months of prior nucleoside analogue treatment were randomized to zidovudine 600 mg/d plus didanosine 400 mg/d with or without nevirapine 400 mg/d. The patients had a mean CD4+ cell count of 138/μL and a median duration of prior therapy of 25 months. According to Dr Pottage, the triple combination was associated with a clear benefit in CD4+ cell count increase and decreases in PBMC viral titer and plasma viral RNA levels. Further studies of the potential clinical benefit of combination treatment including nevirapine are under way.

Delavirdine (a BHAP compound) is another NNRTI with significant promise that is currently being extensively investigated in clinical trials. As with other NNRTIs, resistance to BHAP compounds is rapidly selected for, with resistance in vitro being observed after three to five passages. In early studies, the agent has been well tolerated at doses up to 400 mg tid. Rash has been observed in 27% to 38% of patients, but patients were successfully rechallenged or treated through the effect in all but one case. The agent undergoes hepatic metabolism and exhibits increased clearance in the presence of rifampin and rifabutin. Increased liver function tests have been observed in combination therapy recipients. Early findings with combination therapy including delavirdine have indicated that sustained improvements in immunologic and virologic markers are achieved. Studies of delavirdine monotherapy and combination therapy are ongoing. Studies currently open for enrollment include ACTG 260 and ACTG 261.

In summarizing the current status of NNRTIs, Dr Friedland observed that: (1) three agents (nevirapine, delavirdine, and loviride) are in the advanced stages of clinical development; (2) the pharmacologic and safety profiles of these classes of agent are favorable; (3) although altered drug susceptibility occurs rapidly, improvements in virologic and immunologic markers have been demonstrated with monotherapy and combination therapy; and (4) larger clinical trials will determine whether these agents provide sufficient benefit in terms of viral load reduction and clinical outcome to be clinically useful.

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